Brit. J. Pharmacol. (1962), **19**, 85–98.

STUDIES ON SYMPATHETIC MECHANISMS IN ISOLATED INTESTINAL AND VAS DEFERENS PREPARATIONS

BY

G. A. BENTLEY*

From the Nicholas Institute for Medical and Veterinary Research, Sherbrooke, Victoria, Australia

(*Received February* 14, 1962)

The effects of various drugs which block sympathetic nerves have been studied in Finkleman preparations of rabbit and cat ileum. Contractile responses could be produced in many cases after the normal inhibitory responses to periarterial nerve stimulation had been blocked. In almost all cases this contractile response was abolished by ganglion-blocking drugs. The normal rabbit ileum Finkleman preparation was found to behave differently towards bretylium than do preparations taken from animals treated with reserpine. The block of the guinea-pig isolated hypogastric vas deferens preparation caused by hemicholinium HC-3 was found to be reversed in the presence of noradrenaline, histamine, or 5-hydroxytryptamine, as was the block caused by guanethidine and bretylium. The results are discussed in relation to the Burn-Rand theory of sympathetic nerve mechanism.

In recent years the discovery of three chemically dissimilar groups of drugs that specifically block post-ganglionic sympathetic nerves has led to considerable investigation into the finer details of sympathetic mechanisms. There is evidence to suggest that both reserpine (Burn & Rand, 1957) and guanethidine (Cass, Kuntzman & Brodie, 1960 ; Wylie, 1961 ; McCubbin, Kaneko & Page, 1961) may produce their blocking action by depleting the nerves of noradrenaline. Guanethidine has been shown also to interfere with the release of noradrenaline from sympathetic nerves (Maxwell, Plummer, Povalski & Schneider, 1960). Bretylium, the third specific sympatholytic, appears to act in yet another way, possibly by specifically anaesthetizing sympathetic post-ganglionic fibres (Boura, Copp, Duncombe, Green & McCoubrey, 1960), though this theory has been challenged by Boyd, Chang & Rand (1961).

Burn & Rand (1960) have shown that, when sympathetic nerves have been depleted of their normal noradrenaline content by reserpine treatment, stimulation produces a response that resembles the effects of acetylcholine rather than of noradrenaline. This " sympathetic reversal " has led Burn & Rand to propose a new theory, which postulates that the whole of the autonomic system is basically cholinergic. These authors suggest that when post-ganglionic sympathetic fibres are excited there is a

*Present address: Department of Physiology, Monash University, Clayton, Victoria, Australia.

release of acetylcholine at the terminals. This, they believe, acts as a "trigger," and causes the subsequent release of noradrenaline from storage sites in or around the nerve terminals. However, Gillespie & MacKenna (1961) have brought forward evidence which suggests that, at least in the rabbit colon, the sympathetic reversal brought about by reserpine is due to acetylcholine from parasympathetic fibres, whose effect is "exposed" in the absence of the normal sympathetic response. Day & Rand (1961), however, have more recently examined the reversed responses obtained in rabbit ileum treated with guanethidine and have concluded that the parasympathetic system is not involved in this phenomenon.

In view of these conflicting opinions it seemed important to test the responses of sympathetically innervated organs to various drugs which act on cholinergic and adrenergic mechanisms. For this purpose two isolated organ preparations were used, namely, the Finkleman preparation of rabbit ileum (Finkleman, 1930) and the guinea-pig isolated hypogastric-vas deferens preparation. These were chosen so as to compare inhibitory and excitatory sympathetic responses and the effects of various drugs on them. The hypogastric nerve probably contains only sympathetic nerve fibres (see Gruber, 1933), while the periarterial nerves in the Finkleman preparation appear to contain fibres from both divisions of the autonomic system.

METHODS

Isolated intestine preparations

Most of the experiments reported in this paper were done using the standard Finkleman preparation of rabbit ileum (Finkleman, 1930). Other experiments were done using modified Finkleman preparations, set up with provision for eliciting and recording the peristaltic reflex and for transmural electrical stimulation. Some work was also done using isolated kitten ileum, which was stimulated via the periarterial nerves.

These preparations were mounted on "perspex" holders, suspended in Tyrode solution at 37° C, and aerated with 95% oxygen and 5% carbon dioxide. The periarterial nerves were either clamped to platinum electrodes (insulated to within 7 mm of the tips and covered by a perspex plate), or were attached to "fluid electrodes" similar to those described by Garry & Wishart (1951). The preparations used to record the peristaltic reflex and the effects of transmural electrical stimulation were mounted on a glass J-tube containing a platinum electrode which protruded a few mm into the lumen of the preparation. An indifferent electrode, also of platinum, dipped a few cm below the surface of the Tyrode solution. The periarterial nerves were stimulated through platinum electrodes identical with those used on the conventional Finkleman preparations. The peristaltic reflex was elicited by injecting a small quantity of air (0.4 to 0.8 ml.) from a tuberculin syringe into the tube leading into the lumen of the intestine. The reflex rhythmic changes in pressure so produced were recorded by a lever mounted on a tambour.

The electrical stimulation was provided by a Grass Model S4DR Stimulator. The periarterial nerves were stimulated with square-wave bipolar shocks of 1 msec duration, at a frequency of 30 to 50/sec. (Gillespie & MacKenna (1959) have shown this to be the optimal frequency for stimulating sympathetic fibres, while the parasympathetic fibres respond best to a frequency of 5/sec.) The stimuli were applied for periods of 20 sec every 2.5 to 3 min. The voltage was adjusted so that it produced complete inhibition of the beat of the intestine, with rapid recovery on termination of the stimulus. Where transmural stimulation was used, the shocks were applied at a rate of 5/sec for periods of 5 sec. This caused a brief contraction. When frequencies of 50/sec were used, the initial contraction was followed by a relaxation.

Isolated guinea-pig hypogastric-vas deferens preparations

These were set up in a similar manner to the Finkleman preparations, using the same holders and electrode. The hypogastric nerve was stimulated with square-wave bipolar shocks, delivered at a frequency of 30 to 50/sec, in bursts lasting 5 sec, every 2 min. The voltage was adjusted (unless otherwise stated) to give maximal contractions.

All tracings were recorded on smoked paper using isotonic, frontal writing levers.

Drugs. Serpasil (Ciba) solution of reserpine, Ismelin (Ciba) tablets of guanethidine, nicotine tartrate (British Drug Houses), dimethyl phenyl piperazinium iodide (L. Light), eserine sulphate (T. & H. Smith), acetylcholine (Roche), hemicholinium HC-3 (J. P. Long), and Levophed (Winthrop) brand of (−)-noradrenaline bitartrate were used. The bretylium (as the p-toluene sulphonate) was synthesized in the laboratories of Aspro-Nicholas, England.

Rabbits being treated with reserpine usually received the drug intravenously, either undiluted from the ampoule, or, where the animals seemed dehydrated, the drug was diluted in 6% glucose solution. Cats, and a few of the rabbits, were dosed by intraperitoneal injection. Rabbits received increasing doses. In two cases, doses rising from 0.5 to 3.0 mg/kg/day over 4 days were given by subcutaneous or intraperitoneal routes. All other animals received the drug intravenously, the dose regimes varying between 1 mg/kg/day, rising to 3 mg/kg/day over four days, and 0.3 mg/kg/day, rising to 0.4 mg/kg/day during 15 days. Cats received 3 daily doses of 1 mg/kg, intraperitoneally.

RESULTS

Effects of various drugs on the normal rabbit ileum Finkleman preparation

Electrical stimulation of the periarterial nerves of this preparation was found to produce only inhibition of the beat if an adequate voltage was used. Contractile responses were never observed in untreated preparations, though Finkleman (1930) has reported that sometimes these occur at the beginning of an experiment. At any given voltage, stimuli delivered at a rate of 30 to 50/sec produced larger inhibitions than when given at a rate of 5/sec.

The addition of either eserine (up to 1×10^{-7}) or atropine (1×10^{-6}) to the preparation was found to produce negligible effects on the response to stimulation of the periarterial nerves. Higher concentrations of eserine caused the muscle to go into spasm, and in this case the inhibition was usually diminished. Combinations of atropine (1×10^{-6}) and carbaminoyl choline (2×10^{-6}) were similarly inactive on the response to periarterial nerve stimulation, neither potentiating the effect of submaximal stimuli nor reducing the response to a maximal stimulus. Another group of compounds found to be without any effect on the response to periarterial nerve stimulation were the ganglion-blocking drugs hexamethonium at 1×10^{-4} and pempidine at 1×10^{-5}.

Bretylium. As noted by Boura & Green (1959), the addition of bretylium to the organ bath caused a slow reduction in the response to periarterial nerve stimulation, without any decrease in the response to added noradrenaline. To produce complete block of the nerve, a concentration of 1×10^{-5} was usually needed, though this varied between 5×10^{-6} and 2×10^{-5}. In the early stages of the block, it was sometimes possible to "break through" by raising the voltage. However, after about 20 min exposure to effective concentrations of the compound, no response could be obtained, even when the voltage was increased up to 8 times the original level. At no stage was a contractile response seen, even when these elevated voltages

were used (12 experiments) (Fig. 1). In eight of these experiments, eserine (4×10^{-8} to 1×10^{-7}) was added after the bretylium-induced block was complete, and in three other cases the eserine was added at the same time as the bretylium. In only three of the 11 preparations treated with a combination of bretylium plus eserine were contractile responses seen after blockade of the normal inhibitory response to periarterial nerve stimulation. These contractile responses were small, never increasing the height of the beat by more than 20%, and they occurred only when the stimulus voltage was increased by a factor of 2 to 6 times above the original level.

The block produced by bretylium was not affected by the addition of noradrenaline or adrenaline to the bath in amounts initially producing up to 60% reduction in the height of the beat and acting for 10 to 15 min.

Guanethidine. Guanethidine behaved very similarly to bretylium, but blocked the response to periarterial nerve stimulation at somewhat lower levels. Concentra-

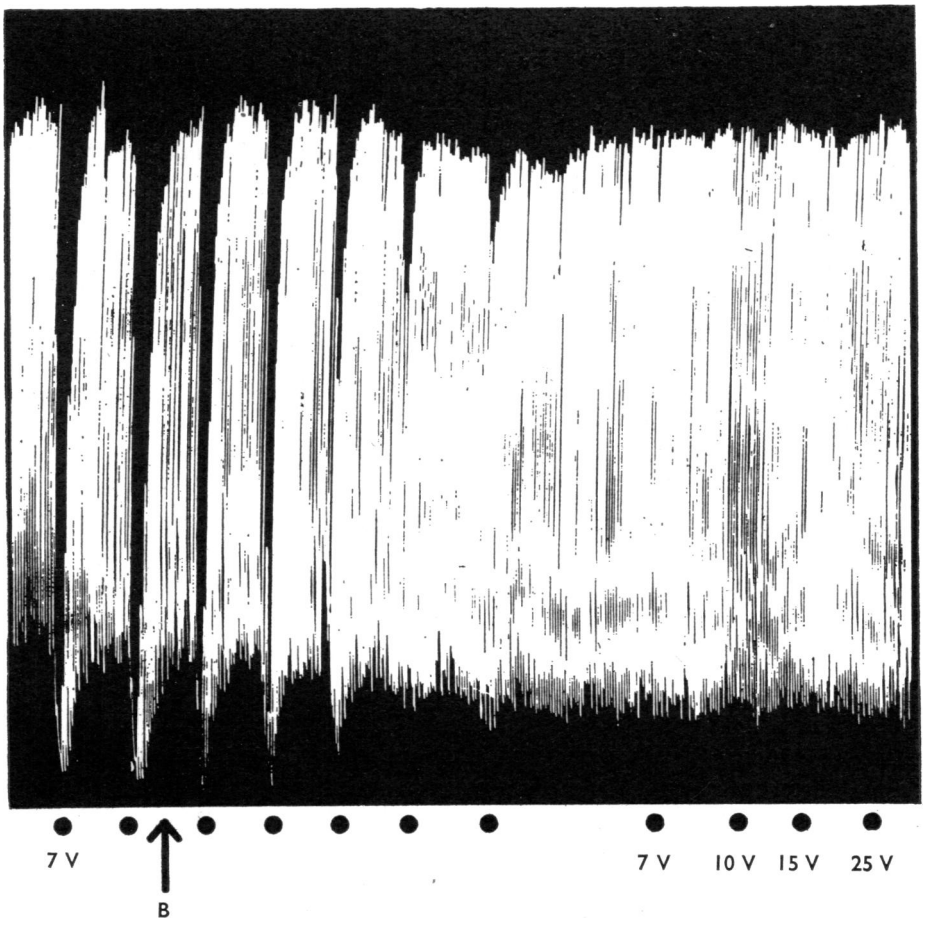

Fig. 1. Finkleman preparation from a normal rabbit. At B, bretylium added to 10^{-5}. Stimuli to periarterial nerves applied at dots.

tions of 2×10^{-6} usually were sufficient completely to abolish the inhibitory response. A second difference from bretylium was also seen, in that small contractile responses occurred in 5 experiments out of 12, when the blocked preparations were stimulated with raised voltage (Fig. 2). When Finkleman preparations were treated with guanethidine plus eserine (2 to 4×10^{-8}) contractile responses were obtained in all

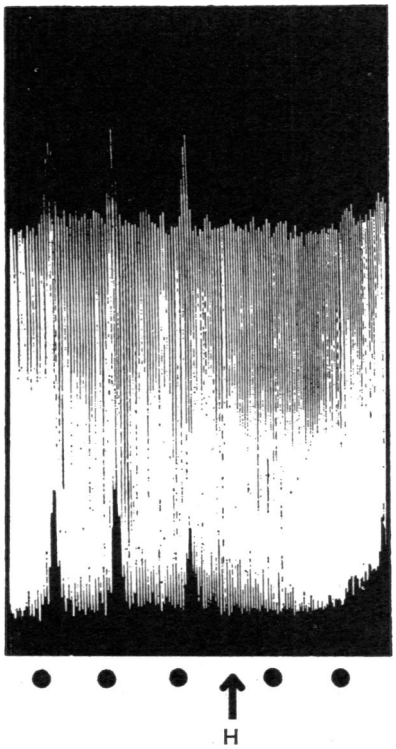

Fig. 2. Finkleman preparation from normal rabbit. Small contractile responses produced by treatment with guanethidine 5×10^{-6} plus eserine 1×10^{-7}. At H, hexamethonium 1×10^{-5} added. Periarterial nerve stimulation applied at dots.

cases (8 experiments). It was immaterial whether the eserine was added together with the guanethidine or after the block was complete. Again, these contractile responses did not increase the height of the beat by more than 20%. They were blocked by hexamethonium 1×10^{-5}.

The guanethidine block, as with that produced by bretylium, was not affected by soaking the tissue in noradrenaline.

Dimethyl phenyl piperazinium. The ganglion-stimulating drug dimethyl phenyl piperazinium was found to have approximately the same potency as guanethidine in blocking the response to periarterial nerve stimulation. At concentrations in the bath of 1 to 4×10^{-6}, a complete block of the response was obtained in 10 to 20 min. In 1 out of 5 experiments a contractile response was seen when the blocked preparation was stimulated with raised voltage.

The block produced by dimethyl phenyl piperazinium was not affected by soaking the preparation in noradrenaline. Unlike either guanethidine or bretylium, dimethyl phenyl piperazinium produced an increased sensitivity to added noradrenaline by a factor of 2 to 3.

Nicotine. Unlike the two other ganglion blocking agents, pempidine and hexamethonium, nicotine sometimes caused some degree of block in the response to stimulation of the periarterial nerves. In 1 out of 8 experiments the block was complete in a concentration of nicotine of 4×10^{-6}, while the response to added noradrenaline was unaffected. In 3 more experiments, a partial block was produced in the presence of nicotine at levels between 4×10^{-6} and 1×10^{-5}.

At no stage was there any suggestion that nicotine increased or prolonged the response to nerve stimulation, even when submaximal stimuli were used.

Hemicholinium HC-3. Since hemicholinium is moderately active in blocking the hypogastric nerve (Chang & Rand, 1960) it was surprising to find that, in 5 experiments, this drug at levels of 1×10^{-4} had absolutely no effect on the response of the Finkleman preparation to stimulation of the periarterial nerves, even when allowed to act for periods of 45 min.

Standard Finkleman preparations taken from rabbits that had received prolonged treatment with reserpine

In all, 15 rabbits have been treated with repeated doses of reserpine. Of these, preparations from only three animals showed contractile responses when stimulated via the periarterial nerves. In all the others, stimulation produced a small inhibition (usually not above 50% of the beat), and this was not increased by raising the voltage. This suggested that, while the stores of noradrenaline in the peripheral nerves were reduced, in most cases there were still small amounts remaining. Six of the incompletely depleted preparations were treated with bretylium at levels of 5×10^{-6} to 2×10^{-5}. In five cases the small inhibitory responses were rapidly blocked, and, as these diminished, contractile responses emerged, until after about 10 min pure contractions were obtained (Fig. 3). In the sixth case, only blockade of the inhibitory responses was seen and no contraction after treatment with bretylium. Guanethidine and dimethyl phenyl piperazinium similarly converted the small inhibition into contractions.

It must be pointed out that where contractile responses were seen in reserpinized preparations (either initially or after treatment with bretylium or guanethidine) the contractions at least doubled the normal height of the beat.

These contractile responses, both spontaneous or after treatment with the blocking drugs, were increased in the presence of eserine (2×10^{-8} to 1×10^{-7}) and were almost always reversibly blocked by ganglionic blocking drugs (hexamethonium 1×10^{-5} to 1×10^{-4}, pempidine 1 to 5×10^{-5}) (Table 1). On the other hand, they were resistant to concentrations of atropine (up to 1×10^{-7}) which completely blocked the response to added acetylcholine. Bretylium, however, had no effect on these contractions, which persisted unchanged in the presence of concentrations of this drug (1 to 2×10^{-5}) which block the inhibitory responses of normal Finkleman prepara-

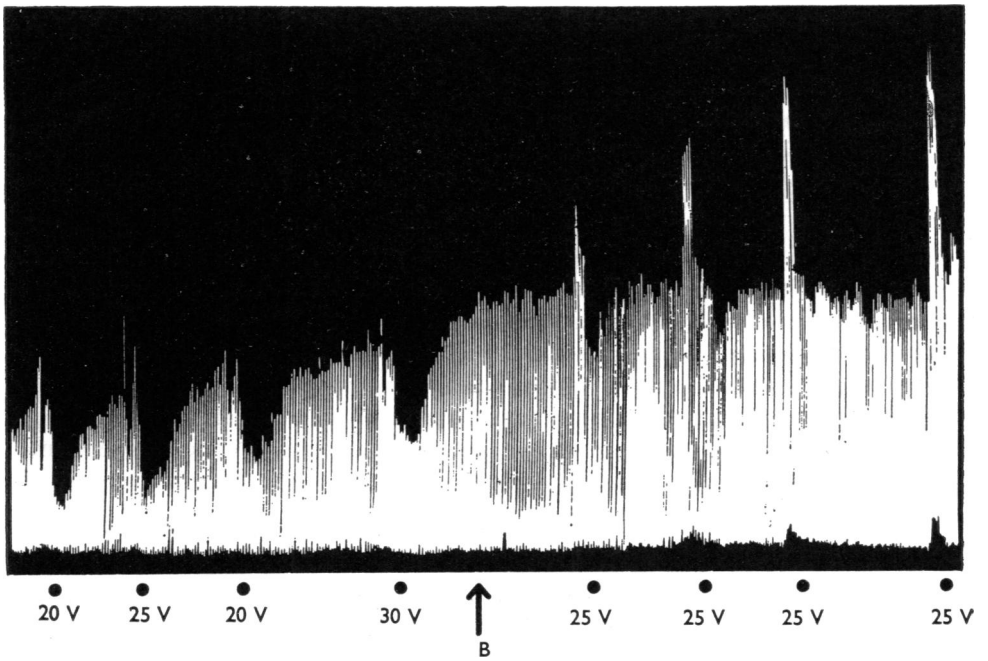

Fig. 3. Finkleman preparation from rabbit treated with reserpine, showing small inhibitory responses not increased by raising voltage. At B, bretylium added to 1×10^{-5}. Periarterial nerve stimulation applied at dots.

TABLE 1

ACTION OF GANGLION-BLOCKING DRUGS ON THE CONTRACTILE RESPONSE OF RABBIT AND CAT ILEUM TREATED WITH SYMPATHOLYTIC DRUGS

Treatment producing contractile response	Ganglion-blocking drug and concentration	Effect on contraction	No. of experiments
(1) *Rabbit ileum*			
Guanethidine plus eserine *in vitro*	Hexamethonium 1×10^{-5}	Blocked	2
Reserpine *in vivo*	Hexamethonium 1×10^{-5}	Halved	2
	Pempidine 1×10^{-6}	Blocked	1
Reserpine *in vivo* plus bretylium *in vitro*	Hexamethonium 1×10^{-5}	Blocked	4
	2×10^{-5}	Not affected	1
	2×10^{-5}	Almost blocked	1
	5×10^{-5}	Blocked	1
	Pempidine 8×10^{-6}	Blocked	1
	1×10^{-5}	Blocked	1
	Nicotine 5×10^{-6}	Blocked	1
Reserpine *in vivo* plus dimethyl phenyl piperazinium *in vitro*	Hexamethonium 5×10^{-5}	Blocked	1
(2) *Cat ileum*			
Reserpine *in vivo*	Hexamethonium 5×10^{-6}	Blocked	1
	1×10^{-5}	Almost blocked	1
	1×10^{-5}	Halved	1
	Pempidine 5×10^{-6}	Blocked	1
	1×10^{-5}	Blocked	1
Bretylium *in vitro*	Hexamethonium 1×10^{-5}	Blocked	1
	2×10^{-5}	Blocked	1
	Nicotine 2×10^{-6}	Blocked	1

tions. Where the preparations from reserpinized animals were still giving small inhibitory responses, bretylium blocked these, as described above, and the resultant contractile response persisted unchanged for several hours in concentrations of 1 to 2×10^{-5} bretylium.

Modified rabbit ileum Finkleman preparations with intralumenal electrode and peristaltic reflex

Gillespie & MacKenna (1961) have suggested that, in the rabbit colon, the sympathetic system may activate parasympathetic fibres at the periphery. It was attempted to investigate this theory in the following manner. After the inhibitory response to periarterial nerve stimulation had been completely blocked by bretylium, these nerves were stimulated with three times the voltage that had previously caused complete inhibition. At the same time, transmural shocks were applied through the intralumenal electrode at a voltage that had previously produced a very small contraction. No potentiation of the response to transmural stimulation was seen by the simultaneous stimulation of the blocked periarterial nerves. The peristaltic reflex was shown at the same time to be completely unaffected by the bretylium.

Other intestinal preparations

Finkleman preparations made from kitten ileum behaved very much as did those from rabbit; they relaxed in response to stimulation of the periarterial nerves. However, unlike rabbit preparations, contractile responses were readily obtained when treatment with bretylium (1×10^{-5}) had blocked the normal inhibitory response, even in the absence of eserine. This contractile response was blocked by hexamethonium (1×10^{-5} to 1×10^{-4}) or pempidine (5×10^{-6} to. 1×10^{-5}) (Table 1). Similarly, guanethidine at 2×10^{-6} rapidly blocked the inhibitory responses and converted these to contractions.

Finkleman preparations from cats receiving repeated injections of reserpine

Cats appear to be more susceptible than rabbits to the depleting action of reserpine. It was found that 3 daily intraperitoneal injections of 1 to 2 mg/kg of reserpine were sufficient to produce contractile responses to stimulation of the periarterial nerves of Finkleman preparations made from their small intestines. These contractile responses were potentiated by eserine (5×10^{-8}) and were blocked by hexamethonium (1×10^{-5}) or pempidine (1×10^{-5}) (Table 1). They were reduced but not blocked by bretylium 1×10^{-5} or guanethidine 2×10^{-6}.

Guinea-pig hypogastric-vas deferens preparations

Bretylium. Stimulation of the hypogastric nerve with supramaximal shocks for a period of 5 to 10 sec produced regular and rapid contractions which relaxed immediately the stimulus was switched off. The addition of bretylium to a level of 1×10^{-5} produced a complete failure of this response in a period of 10 to 20 min. Once the block was established, raising the voltage by a factor of two or three produced no effect. This block persisted for at least 1 hr after the bretylium was washed out. At this stage, addition of noradrenaline 1 to 4×10^{-6}, histamine

4×10^{-6} to 1×10^{-5} or 5-hydroxytryptamine 1 to 2×10^{-5} would partly or completely restore the contractions. The concentrations of these drugs that were used caused only a small rise in the resting tension. After these compounds had been washed out, the response to nerve stimulation rapidly disappeared again (see Fig. 4).

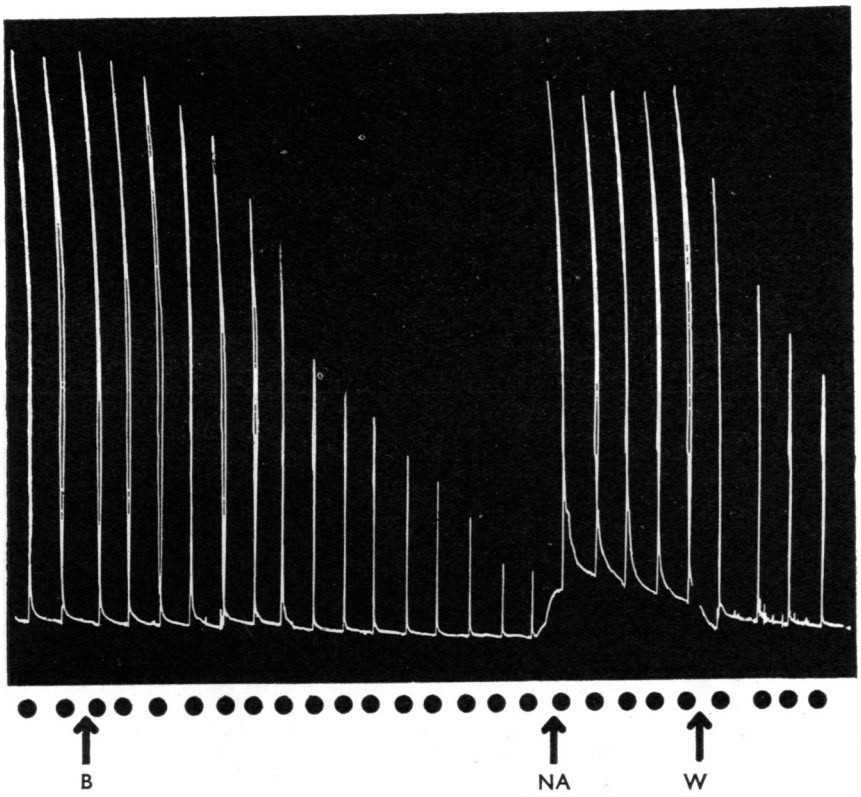

Fig. 4. Guinea-pig hypogastric-vas deferens preparation. At B, bretylium 5×10^{-6} added, and at NA noradrenaline 2×10^{-6} added. At W, bath washed out. Stimuli to hypogastric nerve at dots.

Guanethidine. This compound behaved very similarly to bretylium, but again was more active, producing complete block at levels of 1 to 2×10^{-6}. Once established, this block resisted raised voltage, and persisted for at least 1 hr after washout. As in the case of bretylium, the addition of low concentrations of noradrenaline, histamine or 5-hydroxytryptamine partially or completely reversed the block, and this action rapidly disappeared when these compounds were washed out of the bath.

Eserine. Up to concentrations of 1×10^{-6}, eserine had very little effect on the response to stimulation of the hypogastric nerve, but occasionally caused a small increase in the response to submaximal stimuli.

Carbaminoyl choline. This drug at levels of 1×10^{-7} to 1×10^{-6} was very active in potentiating the response of the preparation to submaximal nerve stimulation. At this level it caused no spontaneous contraction of the muscle.

Nicotine. At concentrations between 5×10^{-7} and 2×10^{-6} nicotine had no effect on the responses to either maximal or submaximal stimulation of the nerve. At levels between 2×10^{-6} and 5×10^{-6} nicotine rapidly blocked the response of the preparation. This block resisted raised voltages, and was not affected by the addition of noradrenaline up to 1×10^{-6}. After the nicotine was washed out, the responses rapidly returned to the original levels.

Dimethyl phenyl piperazinium. This compound also blocked the response to hypogastric nerve stimulation. It was less active on this preparation than on the Finkleman preparation, concentrations of 4 to 6×10^{-6} being necessary to produce complete block. The effect developed slowly and once established persisted for some hours after the drug was washed out of the bath. The block resisted raised voltage (by a factor of 2 or 3), but in the presence of noradrenaline (4×10^{-7}) the contractions in response to stimulation of the nerve returned. After the nor-

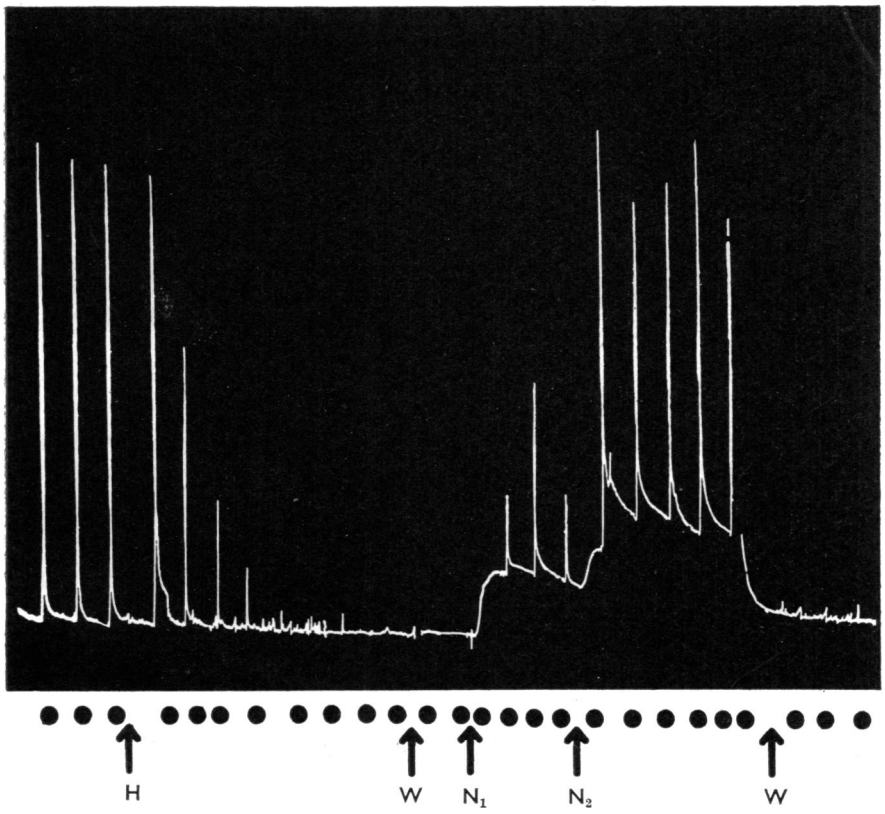

Fig. 5. Guinea-pig hypogastric-vas deferens preparation. At H, hemicholinium 1×10^{-4} added to bath. At W, bath washed out. At N_1, noradrenaline added to $0{\cdot}5 \times 10^{-6}$, at N_2 noradrenaline increased to 1×10^{-6}. Stimuli to hypogastric nerve at dots.

adrenaline was washed out, the responses failed again. The site of the block produced by dimethyl phenyl piperazinium seemed to be presynaptic, since, in the presence of this drug, the contractions produced by added noradrenaline were potentiated.

Hemicholinium. As noted by Chang & Rand (1960), the addition of hemicholinium at levels of 5×10^{-5} to 1×10^{-4} produced a progressive failure of the response to stimulation of the hypogastric nerve. Some reversal of this block could be obtained by the subsequent addition of choline 5×10^{-4}, but a much more rapid and complete restoration of the contractions was obtained in the presence of noradrenaline at levels of 1 to 4×10^{-6} (Fig. 5). Histamine at 1×10^{-5} and 5-hydroxytryptamine at 5×10^{-6} to 1×10^{-5} also reversed the block caused by hemicholinium, which was rapidly re-established when these drugs were washed out of the bath. Hemicholinium had no blocking action against added noradrenaline.

DISCUSSION

The contractile response seen in Finkleman preparations treated with reserpine alone, or reserpine plus bretylium or guanethidine, or with guanethidine plus eserine, might have one of three possible origins. These are (*a*) cholinergic sympathetic fibres (von Euler & Gaddum, 1931), (*b*) the cholinergic "trigger" mechanism postulated by Burn & Rand (1960), or (*c*) parasympathetic fibres in the periarterial nerves. The fact that these contractile responses, however produced, are almost always abolished by ganglion-blocking drugs suggests that the third possibility is the most likely one, and, in rabbit intestine at least, there is no evidence to suggest the presence of a cholinergic trigger. It is puzzling that the present results obtained by the use of ganglion-blocking drugs are completely at variance with the report of Day & Rand (1961). These workers produced contractile responses in rabbit ileum Finkleman preparations by treatment with guanethidine, and claimed that these contractions were resistant to levels of hexamethonium up to 1×10^{-4}. No explanation can be advanced for this discrepancy.

In contrast with reserpine, bretylium alone has never been observed to produce a contractile response. Day & Rand (1961) have also noted this fact, and Burn & Rand (1960) have also noted a similar effect of bretylium on the cat nictitating membrane. In this organ, they showed that the contractions caused by stimulation of the cervical sympathetic nerve were completely blocked by this drug. On the other hand, after treatment with reserpine, they noted that small contractions could still be elicited but that these were blocked by atropine.

Hence it would seem that, in these two tissues, sympathetic blockade by reserpine reveals cholinergic responses, while, after bretylium alone, these are not seen. One possible explanation for this might have been that bretylium abolished the cholinergic trigger postulated by Burn & Rand. However, this theory is not tenable, since the contractile response seen in Finkleman preparations taken from reserpinized rabbits is not affected by bretylium. For the same reason, the lack of contractile responses after bretylium cannot be due to a ganglion-blocking action of this drug, though Gertner & Romano (1961) have shown that bretylium has this action.

It might be suggested that bretylium alone is incapable of completely suppressing the release of noradrenaline and that a sufficient amount is released after block exactly to counteract the contraction caused by the cholinergic trigger. The combination of noradrenaline depletion with reserpine plus blockade by bretylium would in this case seem to be necessary to expose a contractile response. However, this explanation also seems unlikely, for two reasons. Firstly, it is hard to imagine that after bretylium blockade, which will resist up to eight times the previously effective voltage, there still remains some hidden release of noradrenaline which always exactly neutralizes the effects of the cholinergic trigger. Secondly, after partial reserpinization, when there is still some small inhibitory response, this is often preceded by a small contraction. When bretylium is then added, the contractions become larger as the inhibitory response is blocked.

A more likely explanation for the lack of contractile responses in preparations blocked by bretylium (and for the small size of the contractions obtained with guanethidine plus eserine) may be connected with the ability of noradrenaline to suppress ganglionic transmission. Costa *et al.* (1961) have shown that perfusion of the superior cervical ganglion of cats with noradrenaline raises the threshold to preganglionic stimulation, and that, as the noradrenaline content of the ganglion is reduced by perfusion with increasing concentrations of reserpine, the threshold to preganglionic stimulation becomes lower than normal. It seems reasonable to expect, therefore, that in a reserpinized Finkleman preparation with depleted noradrenaline stores the conduction across parasympathetic ganglia would be more active, and thus contractile responses to stimulation of the periarterial nerves would be larger when the sympathetic responses had been completely blocked.

This ability of reserpine, not only to deplete post-ganglionic sympathetic fibres of noradrenaline but also to enhance ganglionic transmission, points to the need for caution in the interpretation of results obtained from reserpinized preparations.

Other findings reported in this paper cast further doubts on the validity of the Burn-Rand theory. If such a cholinergic trigger mechanism existed, it might be expected that eserine or nicotine or a combination of atropine plus carbaminoyl choline would potentiate sympathetic responses. In fact, this does not happen, and nicotine may reduce or block both the inhibitory response in the intestine and the contractile response of the vas deferens.

The experiments of Chang & Rand (1960) used by them as evidence to support the Burn-Rand theory can be interpreted in other ways. These workers used hemicholinium, which is known to block the synthesis of acetylcholine in sympathetic ganglia (McIntosh *et al.*, 1956). Chang & Rand believed that they had demonstrated a cholinergic step in sympathetic transmission when they showed that hemicholinium blocked the hypogastric nerve and that this block was reversed by choline. Hemicholinium has recently been shown to have a post-synaptic blocking action against acetylcholine (Thies & Brooks, 1961 ; Martin & Orkand, 1961), although it does not block the response of the vas deferens to added noradrenaline (see Fig. 5). In the present paper, it was shown that the block of the hypogastric nerve by hemicholinium was reversed by various substances which, in higher concentrations, cause the vas deferens to contract. These were noradrenaline, histamine and 5-hydroxytryptamine.

Since these compounds almost certainly act post-synaptically, it seems inadmissible to suggest that hemicholinium blocks the nerve by an action on a postulated cholinergic trigger. It is interesting to note that hemicholinium in concentrations of 1×10^{-4} caused no block of the inhibitory response to periarterial nerve stimulation in the Finkleman preparation. This is consistent with the findings of Gardiner & Thompson (1961), who found that hemicholinium was similarly inactive in blocking the response of the cat nictitating membrane to stimulation of the cervical sympathetic nerves.

Bretylium, guanethidine and dimethyl phenyl piperazinium also block the hypogastric nerve, and, as with hemicholinium, this block may be reversed by low concentrations of noradrenaline, histamine or 5-hydroxytryptamine. This finding suggests that bretylium does not exert its blocking action by a local anaesthetic action on the nerve, as suggested by Boura & Green (1959). Since the response of the vas deferens to stimulation of the blocked hypogastric nerve is restored by such a variety of compounds, the possibility arises that normally there may be a continual slow release of noradrenaline from sympathetic nerve endings, whose function is to keep the post-synaptic membrane in a partly excited state, so that when the nerve impulse arrives at the synapse the post-synaptic membrane is readily depolarized. Burnstock & Holman (1962) have presented evidence indicating a continuous "resting" discharge of some post-synaptic excitatory substance from the hypogastric nerve.

It must be concluded that, in the present study, no evidence has been found that suggests the presence of a cholinergic step in sympathetic conduction in either the guinea-pig hypogastric nerve or in the periarterial nerves of the rabbit or kitten ileum. The present results neither support nor disagree with the suggestion of Gillespie & MacKenna (1961) that sympatheic nerves may activate the parasympathetic system at the periphery.

REFERENCES

BOURA, A. L. A. & GREEN, A. F. (1959). The actions of bretylium: adrenergic neurone blocking and other effects. *Brit. J. Pharmacol.*, **14**, 536–548.

BOURA, A. L. A., COPP, F. C., DUNCOMBE, W. G., GREEN, A. F. & MCCOUBREY, A. (1960). The selective accumulation of bretylium in sympathetic ganglia and their postganglionic nerves. *Brit. J. Pharmacol.*, **15**, 265–270.

BOYD, H., CHANG, V. & RAND, M. J. (1961). The local anaesthetic activity of bretylium in relation to its action in blocking sympathetic responses. *Arch. int. pharmacodyn.*, **131**, 10.

BURN, J. H. & RAND, M. J. (1957). Reserpine and noradrenaline in artery walls. *Lancet*, **ii**, 1097.

BURN, J. H. & RAND, M. J. (1960). Sympathetic postganglionic cholinergic fibres. *Brit. J. Pharmacol.*, **15**, 56–66.

BURNSTOCK, G. & HOLMAN, M. E. (1962). Spontaneous potentials at sympathetic nerve endings in smooth muscle. *J. Physiol. (Lond.)*, **160**, 446–460.

CASS, R., KUNTZMAN, R. & BRODIE, B. B. (1960). Norepinephrine depletion as a possible mechanism of action of guanethidine (SU 5864), a new hypotensive agent. *Proc. Soc. exp. Biol. med. Sci.*, **103**, 871.

CHANG, V. & RAND, M. J. (1960). Transmission failure in sympathetic nerves produced by hemicholinium. *Brit. J. Pharmacol.*, **15**, 588–600.

COSTA, E., REVZIN, A. M., KUNTZMAN, R., SPECTOR, S. & BRODIE, B. B. (1961). Role for ganglionic norepinephrine in sympathetic synaptic transmission. *Science*, **133**, 1822.

DAY, M. D. & RAND, M. J. (1961). Effect of guanethidine in revealing cholinergic sympathetic fibres. *Brit. J. Pharmacol.*, **17**, 245–260.

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

GARDINER, J. E. & THOMPSON, J. W. (1961). Lack of evidence for a cholinergic mechanism in sympathetic transmission. *Nature* (*Lond.*), **191**, 86.

GARRY, R. C. & WISHART, M. (1951). Fluid electrodes with a rubber diaphragm. *J. Physiol.* (*Lond.*), **115**, 61*P*.

GERTNER, S. B. & ROMANO, A. (1961). Action of guanethidine and bretylium on ganglionic transmission. *Fed. Proc.*, **20**, 319.

GILLESPIE, J. S. & MACKENNA, B. R. (1961). The inhibitory action of the sympathetic nerves on the smooth muscle of the rabbit gut, its reversal by reserpine and restoration by catechol amines and by DOPA. *J. Physiol.* (*Lond.*), **156**, 17–34.

GRUBER, C. M. (1933). Autonomic innervation of the genito-urinary system. *Physiol. Rev.*, **13**, 497–609.

MCCUBBIN, J. W., KANEKO, Y. & PAGE, I. H. (1961). The peripheral cardiovascular actions of guanethidine in dogs. *J. Pharmacol. exp. Therap.*, **131**, 346.

MCINTOSH, F. C., BIRKS, R. I. & SASTRY, P. B. (1956). Pharmacological inhibition of acetylcholine synthesis. *Nature* (*Lond.*), **178**, 1181.

MACKENNA, B. R. & GILLESPIE, J. S. (1959). The effect of reserpine on the response of the rabbit ileum and colon to stimulation of their extrinsic nerves *in vitro*. *J. Physiol.* (*Lond.*), **147**, 31*P*.

MARTIN, A. R. & ORKAND, R. K. (1961). Postsynaptic action of HC-3 on neuromuscular transmission. *Fed. Proc.*, **20**, 579.

MAXWELL, R. A., PLUMMER, A. J., POVALSKI, H. & SCHNEIDER, F. (1960). Concerning a possible action of guanethidine (SU5864) in smooth muscle. *J. Pharmacol. exp. Therap.*, **129**, 24.

THIES, R. E. & BROOKS, V. B. (1961). Postsynaptic neuromuscular block produced by hemicholinium No. 3. *Fed. Proc.*, **20**, 569.

VON EULER, U. S. & GADDUM, J. H. (1931). Pseudomotor contractures after degeneration of facial nerve. *J. Physiol.* (*Lond.*), **73**, 54–60.

WYLIE, D. W. (1961). Augmentation of the pressor response to guanethidine by inhibition of catechol o-methyltransferase. *Nature* (*Lond.*), **189**, 490.