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THE CHOLINERGIC BLOCKING ACTION OF ADRENERGIC BLOCKING AGENTS IN THE PHARMACOLOGICAL ANALYSIS OF AUTONOMIC INNERVATION

BY

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The adrenergic blocking agents tolazoline, phentolamine, piperoxan, yohimbine, phenoxybenzamine, bretylium and guanethidine block the excitatory actions both of cholinergic nerves and of added acetylcholine on a variety of vertebrate smooth muscle preparations. These cholinergic blocking actions often occurred with concentrations lower than those required to block the response of the guinea-pig vas deferens to stimulation of the adrenergic hypogastric nerve. The anti-acetylcholine activities of these drugs have been studied in detail, using the guinea-pig rectum and the toad bladder as test organs. In preparations sensitive to eserine, the anticholinesterase actions of the drugs competed with their anti-acetylcholine actions, so that either potentiation or block of responses to acetylcholine and to cholinergic nerve stimulation occurred with different concentrations. The responses of the toad bladder to acetylcholine were not potentiated by eserine. This enabled the antagonism of acetylcholine by the anti-adrenergic drugs to be estimated without interference from their anticholinesterase activity. When blocking activity was assessed on guinea-pig rectum previously treated with dyflos, the results were qualitatively similar to those on the toad bladder. Phenoxybenzamine often completely blocks responses both to added acetylcholine and to cholinergic nerve stimulation in concentrations less than those required to block adrenergic nerves. Guanethidine and piperoxan also show strong cholinergic blocking activity. Bretylium, yohimbine, tolazoline and phentolamine were less potent. However, in concentrations required to block the effect on the vas deferens of hypogastric nerve stimulation, these drugs at least halved the effects of acetylcholine and often of cholinergic nerve stimulation. It is concluded that these adrenergic blocking agents cannot be used to distinguish conclusively between adrenergic and cholinergic nerves. For reliable analysis of autonomic innervation, the substances released upon nerve stimulation must be identified by specific biochemical techniques or bioassay.

During studies of the comparative physiology of the vertebrate autonomic nervous system (Burnstock, O'Shea & Wood, 1963 ; Burnstock & Campbell, 1963), we were disturbed by the number of smooth muscle preparations in which nervously mediated contraction was blocked both by low concentrations of atropine and by many adrenergic blocking agents. Thus it seemed impossible to distinguish pharmacologically between adrenergic and cholinergic nerves. Nickerson (1949) has already drawn attention to the lack of specificity of drugs which block responses of the sympatho-adrenal system. Anti-adrenergic agents have been said to have activities

like acetylcholine (Gowdey, 1948 ; Ahlquist, Huggins & Woodbury, 1947), atropine (Ross, 1936 ; Kosterlitz & Lees, 1961), anticholinesterases (Boyd, Chang & Rand, 1960) and antihistamines (Gowdey, 1948 ; Stone & Loew, 1952).

The experiments described in this paper were designed to investigate the acetylcholine blocking activity of a number of anti-adrenergic agents, and to determine the limitations of their use as tools for the identification of the transmitters involved in the mediation of excitatory responses to nerve stimulation. A short account of this work has been given (Boyd, Burnstock, Campbell, Jowett, O'Shea & Wood, 1962).

METHODS

Four of the autonomic nerve-smooth muscle preparations used in this investigation have been selected to illustrate the results which were essentially similar for all the preparations examined. These preparations, in each of which nerve stimulation causes contraction, have been classified, mainly on the evidence of analytical pharmacology, as follows:

(1) Adrenergically innervated: guinea-pig hypogastric nerve-vas deferens (Huković, 1961 ; Boyd *et al.*, 1960).

(2) Cholinergically innervated: guinea-pig pelvic nerve-rectum (Boyd & Burnstock, unpublished) similar to that described for the rabbit (Garry & Gillespie, 1955); toad "pelvic nerve"-bladder (Burnstock *et al.*, 1963); possum (*Pseudocheirus peregrinus*) "pelvic nerve"-bladder (Burnstock & Campbell, 1963).

The following preparations were also used: toad rectum, toad lung, lizard lung, guinea-pig bladder, toad stomach, toad intestine, lizard bladder and pigeon terminal colon.

All preparations were suspended in 50 ml. baths and the nerves electrically stimulated with a rectangular wave stimulator (Grass), with supramaximal pulse strength and with controlled frequency and duration. Drugs, dissolved either in isotonic saline or in distilled water, were added in volumes no greater than 1 ml. The preparations from cold and warm blooded animals were maintained as follows:

Toads and lizards, at 20 to 23° C in a solution containing (g/l.) NaCl 7.2 ; KCl 0.28 ; $NaHCO_3$ 0.2 ; NaH_2PO_4 0.01 ; $CaCl_2$ 0.12 ; glucose 2.00 ; bubbled with air.

Mammals (marsupial and placental), at 29 to 30° C in modified Krebs solution (Bülbring, 1953), bubbled with 95% oxygen and 5% carbon dioxide.

Acetylcholine contracted every preparation, and concentrations were used the responses to which could be blocked completely by 10^{-7} g of atropine/ml. (bath concentration) or weaker, so that any nicotinic action of acetylcholine was unlikely.

Experiments designed to elucidate the relationship between the concentration of an adrenergic blocking agent and its potency in antagonizing the action of a fixed concentration of acetylcholine were performed both with the toad isolated bladder and with the guinea-pig isolated rectum. Test concentrations of acetylcholine were submaximal and on the steep section of the dose/response curve, being 10^{-7} g/ml. for the toad bladder and 2×10^{-8} to 10^{-7} g/ml. for the guinea-pig rectum. After control contractions had been obtained, the drug under investigation was added to the perfusing fluid to maintain a constant concentration in contact with the muscle. The test concentration of acetylcholine was applied for 2 min at 10 min intervals during the period of 1 hr following first application of the adrenergic blocking agent. A fresh preparation was used for each concentration of blocking drug. In some experiments, various concentrations of acetylcholine (all on the steep section of the dose/response curve) were tested against a fixed concentration of adrenergic blocking agent.

The relative effects of the adrenergic blocking agents on responses to nerve stimulation and to added acetylcholine were also investigated. The response to acetylcholine was tested before application of the blocking drug, and again when maximal block to nerve stimulation had been achieved.

The drugs were: acetylcholine chloride (Roche); atropine sulphate (D.H.A.); tolazoline hydrochloride (Priscol, 2-benzyliminazoline; Ciba); phentolamine methane sulphonate (Rogitine, Regitine; N.B.C.); yohimbine hydrochloride (N.B.C.); piperoxan (933F, 2-piperidinomethyl-benzo-1:4-dioxan; May & Baker); phenoxybenzamine hydrochloride (Dibenzyline; S.K. & F.); guanethidine pure substance (Ciba); and bretylium tosylate (Darenthin; Wellcome Research Laboratories). Concentrations of these drugs, with the exception of guanethidine, refer to the salts.

RESULTS

The concentrations of adrenergic blocking agents necessary to block responses to adrenergic nerve stimulation usually completely or partially blocked responses

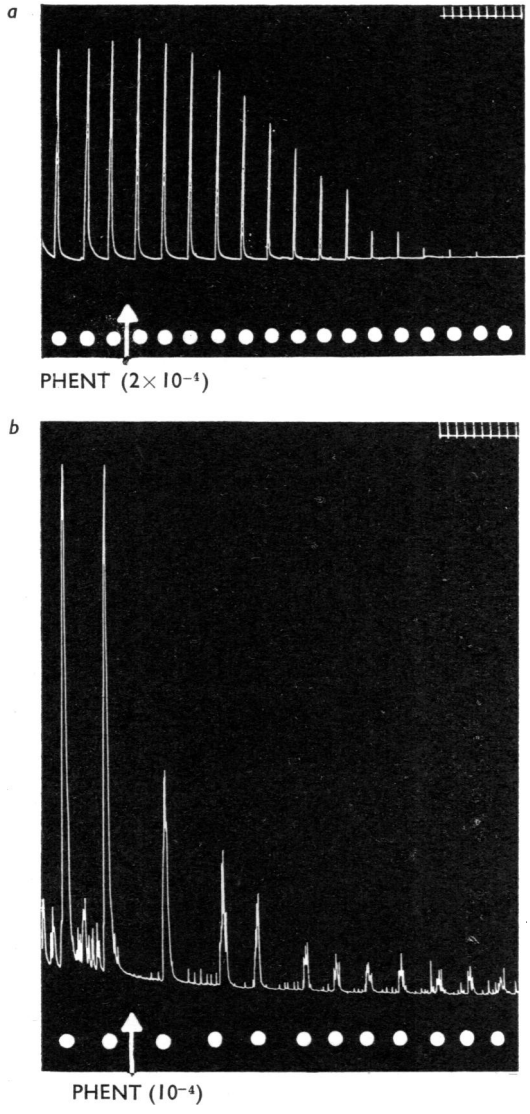

Fig. 1. The effects of the adrenergic blocking agent phentolamine (PHENT) on the responses of two smooth muscle preparations to nerve stimulation (○). (a) Adrenergically innervated preparation: guinea-pig hypogastric nerve-vas deferens. (b) Cholinergically innervated preparation: guinea-pig pelvic nerve-rectum. Bath concentrations in g/ml. Time marker, 1 min intervals.

to cholinergic nerve stimulation (for example, Fig. 1). This effect may be due to an " atropine-like " action of the adrenergic blocking agents or to an action on the nerve. The actions of the adrenergic blocking agents against added acetylcholine were therefore investigated.

The effects of adrenergic blocking agents on responses to acetylcholine

The concentration of acetylcholine used in each experiment was taken from the steepest section of the dose/response curve, so that any potentiation or reduction of its action would be clearly seen. The degree of block of the response to acetylcholine by an adrenergic blocking agent always depended upon the relative concentrations of the two drugs. However, no detailed study was made to determine whether the block of the response to acetylcholine by each drug was competitive and hence strictly " atropine-like."

A study of the relationship between the concentration of the adrenergic blocking agent and its effect on the size of the responses of the toad bladder and guinea-pig rectum to added acetylcholine during a period of 1 hr revealed differences in mechanisms and potencies. On the toad bladder, phenoxybenzamine, guanethidine and piperoxan showed greater acetylcholine blocking activities than did tolazoline, phentolamine, yohimbine and bretylium. The effect of these drugs on the response to acetylcholine applied 10 min later is shown in Fig. 2a. In concentrations of

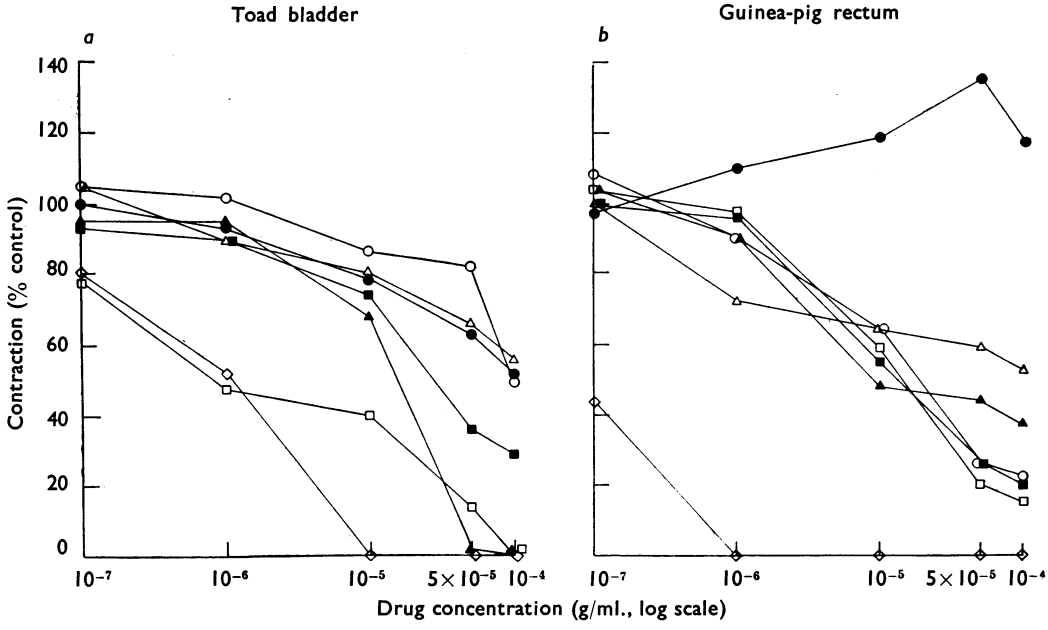

Fig. 2. Dose/response curves for the acetylcholine blocking activity of some adrenergic blocking agents 10 min after their application, on (a) the isolated toad bladder and (b) the isolated guinea-pig rectum. ◇=Phenoxybenzamine; □=guanethidine; ▲=piperoxan; ■=bretylium; ●=tolazoline; △=yohimbine; ○=phentolamine.

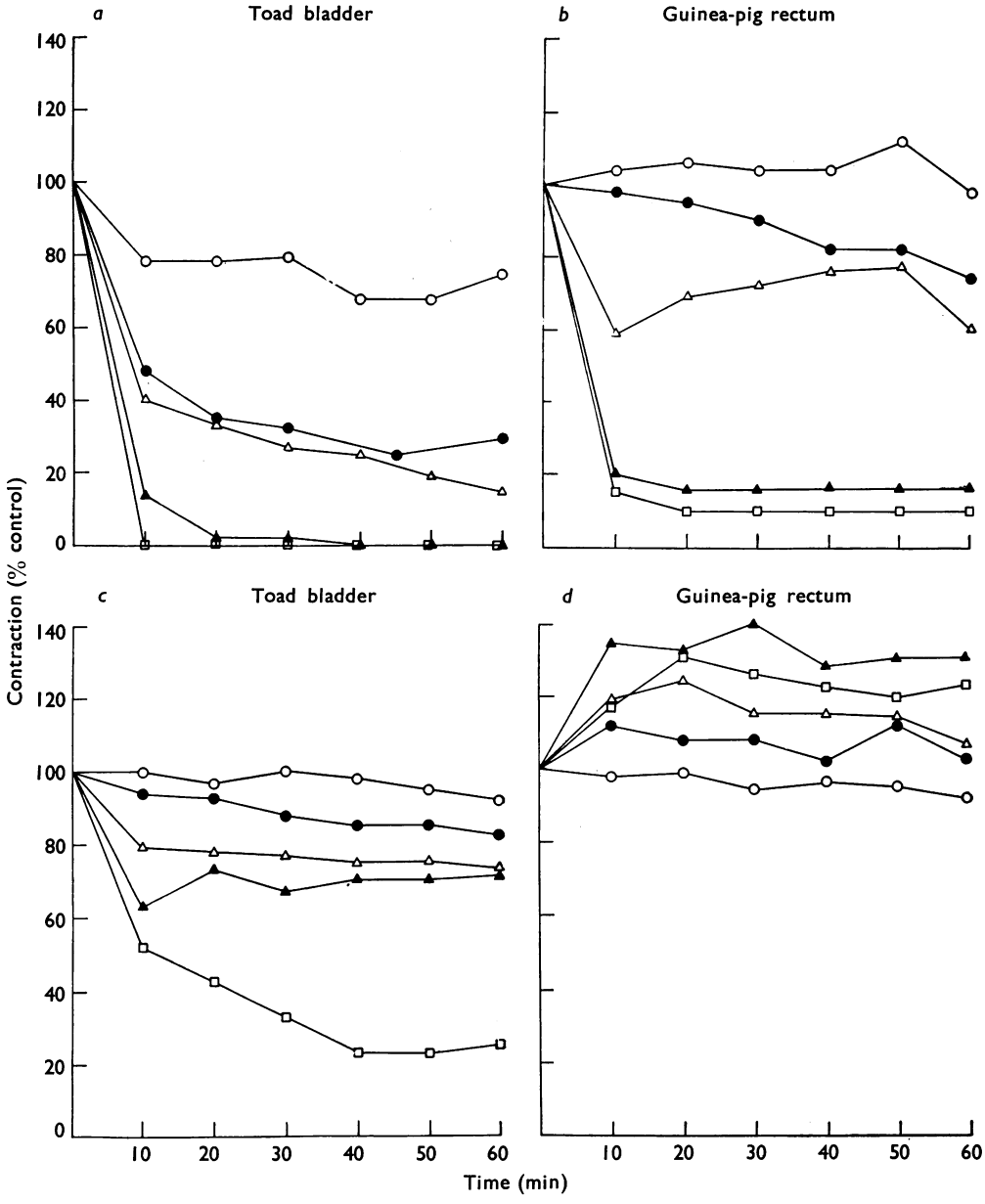

Fig. 3. Actions of guanethidine on (a) the toad bladder and (b) the guinea-pig rectum and of tolazoline on (c) the toad bladder and (d) the guinea-pig rectum in the following concentrations (g/ml.): ○ $=10^{-7}$; ● $=10^{-6}$; △ $=10^{-5}$; ▲ $=5\times10^{-5}$; □ $=10^{-4}$.

10^{-4} g/ml. they had all caused at least 65% block after 30 min, at which time some degree of block was also seen with all the weaker concentrations. On the guinea-pig rectum, phenoxybenzamine caused greater block of acetylcholine than on the toad bladder. On the other hand, tolazoline potentiated the acetylcholine responses in all concentrations tested. The remaining drugs blocked at high concentrations and somewhat potentiated in low concentrations (Fig. 2b).

The difference in patterns of activity on the toad and guinea-pig preparations was most striking with guanethidine and tolazoline (Fig. 3). On the toad bladder, within 40 min, guanethidine (5×10^{-5} g/ml.) caused complete block (Fig. 3a); tolazoline decreased the response to acetylcholine by 30% in a concentration of 5×10^{-5} g/ml. and by 75% at 10^{-4} g/ml. (Fig. 3c). Neither drug caused potentiation at any concentration. On the guinea-pig rectum, however, guanethidine (10^{-7} g/ml.) potentiated the response to added acetylcholine, and even 10^{-4} g/ml. caused only 90% block of the response to added acetylcholine (Fig. 3b). Tolazoline caused potentiation at all concentrations except 10^{-7} g/ml. (Fig. 3d).

The response of the guinea-pig rectum to acetylcholine, unlike that of the toad bladder (Burnstock *et al.*, 1963), is potentiated by eserine. Some of the agents used in these experiments, particularly tolazoline, have an eserine-like activity (Boyd *et al.*, 1960). The difference in the actions of these substances on the two preparations may thus be due to an interplay between their anticholinesterase and anti-acetylcholine actions. This hypothesis was tested by repeating the experiments with tolazoline, guanethidine and yohimbine on guinea-pig rectum which had been previously treated with dyflos using the technique described by Blaber & Cuthbert

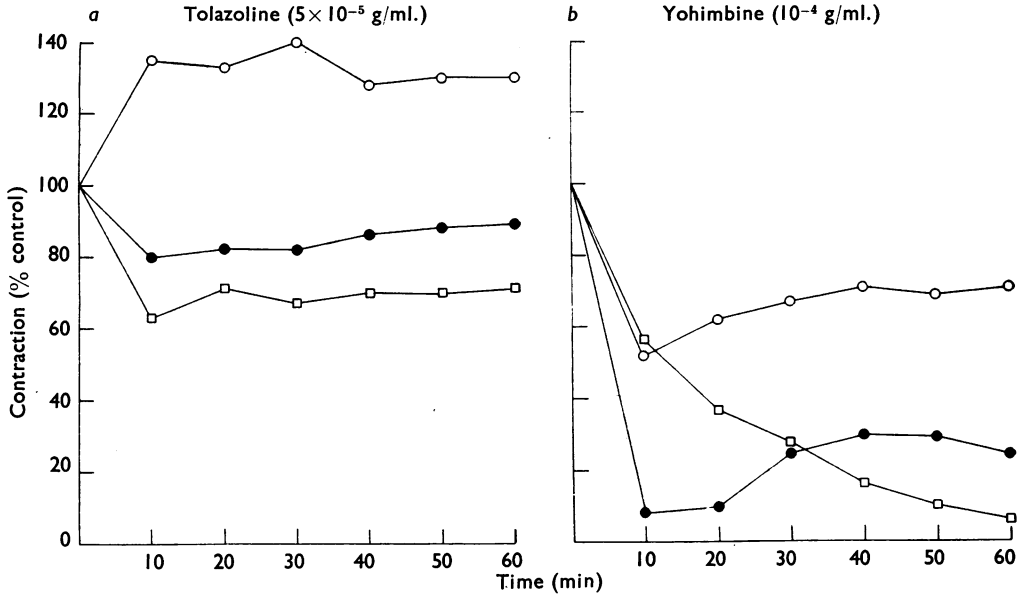

Fig. 4. The effects of (a) tolazoline (5×10^{-5} g/ml.) and (b) yohimbine (10^{-4} g/ml.) on the responses of the normal guinea-pig rectum (○), the dyflos-treated guinea-pig rectum (●), and the toad bladder (□), to applied acetylcholine.

(1961). Under these conditions, the pattern of blocking activity for each of these three drugs was similar to that seen with the toad bladder. For example, the potentiation with tolazoline (5×10^{-5} g/ml.) on the normal guinea-pig rectum was converted to a block on the preparation treated with dyflos, comparable to the 30% block seen with the toad bladder (Fig. 4a). Similarly the blocking activity of yohimbine (10^{-4} g/ml.) was greater on the guinea-pig rectum which had been previously treated with dyflos (Fig. 4b) than on the untreated rectum. The blocking activity of these drugs on the toad bladder was not appreciably affected by treatment with dyflos.

The reduction by the various agents of the response to acetylcholine of the toad bladder should thus give a valid picture of the strength of their acetylcholine antagonism without interference from their anticholinesterase actions. Over the range of test concentrations, the relative potencies remained constant and could be

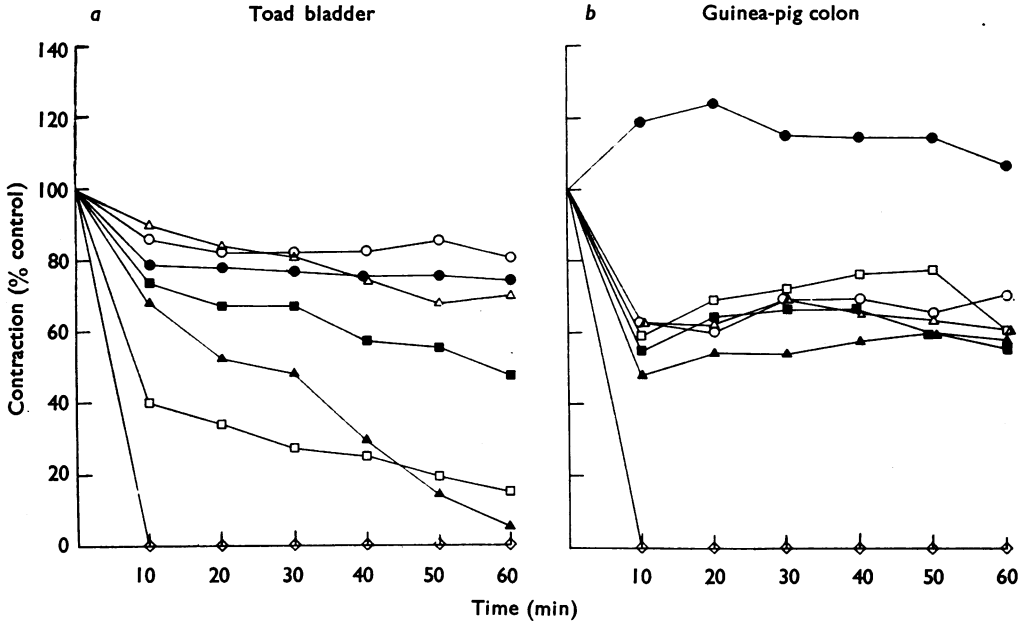

Fig. 5. The effects of adrenergic blocking agents (10^{-5} g/ml.) on the responses of (a) the isolated toad bladder and (b) the isolated guinea-pig rectum to applied acetylcholine over a period of 60 min. ◇=Phenoxybenzamine; ▲=piperoxan; □=guanethidine; ■=bretylium; △=yohimbine; ●=tolazoline; ○=phentolamine.

seen most clearly at a concentration of 10^{-5} g/ml. (Fig. 5a). After 30 min, the anti-adrenergic agents showed the following decreasing order of acetylcholine blocking potency: phenoxybenzamine, guanethidine, piperoxan, bretylium, tolazoline, yohimbine and phentolamine. It should be noted that the concentration of 10^{-5} g/ml. was in all instances weaker than that required to block the response of the guinea-pig vas deferens to stimulation of the adrenergic hypogastric nerve.

With the guinea-pig rectum a different pattern of activity was seen (Fig. 5b). The drugs fall into three groups which appear to reflect an interplay between their anti-acetylcholine and anticholinesterase actions:

(1) Those whose anticholinesterase activity is very strong in comparison with their antagonism of acetylcholine, for example, tolazoline.

(2) Those whose two actions are more equally balanced but whose blocking action usually predominates, especially at higher concentrations, for example, bretylium, piperoxan, yohimbine, phentolamine and guanethidine.

(3) Those for which antagonism of acetylcholine predominates, completely masking their anticholinesterase action, for example, phenoxybenzamine.

The actions of the adrenergic blocking agents on the responses to acetylcholine of every other preparation used varied according to the sensitivity to eserine of the preparation, and in all experiments were qualitatively similar to those actions described above.

Effects of adrenergic blocking agents on responses to nerve stimulation and to applied acetylcholine

The antagonism of acetylcholine and the anticholinesterase activity of the adrenergic blocking agents described above led us to study the relative effects of these agents on responses to nerve stimulation and to acetylcholine. In this way it was hoped to define their use as analytical tools.

Adrenergically innervated preparation: guinea-pig hypogastric nerve-vas deferens

The concentrations of adrenergic blocking drugs required to block the response of the vas deferens to hypogastric nerve stimulation are shown in Table 1A. They are similar to those needed to block other adrenergic nerve-smooth muscle junctions (Varagić, 1956; Brandon & Rand, 1961). After nerve block was complete, the response to added acetylcholine was variable. It was always potentiated by tolazoline (Fig. 6a), but yohimbine, bretylium and guanethidine (Fig. 7a) could either potentiate or partially block it (Fig. 7b). Phenoxybenzamine completely blocked it in concentrations which did not affect the response to nerve stimulation (Fig. 6b). The effects of piperoxan were difficult to assess as this drug caused marked spontaneous activity of the vas deferens in a concentration lower than that required to block the nerve.

With concentrations of drugs lower than those shown in Table 1, potentiation of the response to nerve stimulation was often seen. Since the effects of acetylcholine and adrenergic nerve stimulation on this preparation are potentiated by eserine, the potentiation by the adrenergic blocking agents of the responses of the vas deferens may be due to their anticholinesterase actions (Boyd *et al.*, 1960). The potentiating effects of anticholinesterases at this and other adrenergic nerve-smooth muscle junctions have been further discussed by Burn (1961).

Cholinergically innervated preparations

The effects of the anti-adrenergic agents on responses to nerve stimulation and to acetylcholine were similar (Table 1B).

TABLE I

EFFECTS OF ADRENERGIC BLOCKING AGENTS ON THE RESPONSES OF SMOOTH MUSCLE TO NERVE STIMULATION (N) AND TO ADDED ACETYLCHOLINE (Ach)

C=concentration of adrenergic blocking agent (g/ml.). +++=complete block. ++=>50% block. +=<50% block. P=potentiation. 0=no effect

	Postsynaptic blocking agents															Presynaptic blocking agents					
	Tolazoline			Phentolamine			Piperoxan			Yohimbine			Phenoxybenzamine			Bretylium			Guanethidine		
	C	N	Ach	C	N	Ach	C	N	Ach	C	N	Ach	C	N	Ach	C	N	Ach	C	N	Ach
A: Adrenergically innervated preparation																					
Guinea-pig hypogastric nerve-vas deferens	5×10^{-4}	+++	P	10^{-4}	+++	++	2×10^{-4}	++	+/P	5×10^{-5}	+++	+/P	5×10^{-4}	+++	+++	10^{-4}	+++	+/P	5×10^{-5}	+++	+/P
B: Cholinergically innervated preparations																					
Guinea-pig pelvic nerve-rectum	5×10^{-4}	++	+	10^{-4}	+++	+++	2×10^{-4}	+++	+++	5×10^{-5}	+++	++	10^{-5}	+++	+++	10^{-4}	++	+	5×10^{-5}	++	+++
Toad pelvic nerve-bladder	5×10^{-4}	+++	++	10^{-4}	+++	++	8×10^{-5}	+++	+++	5×10^{-5}	+++	+/++	5×10^{-5}	+++	+++	10^{-4}	+++	++	5×10^{-5}	++	+++
Possum pelvic nerve-bladder	5×10^{-4}	++/P	++/P	10^{-4}	++	++	2×10^{-4}	++	+++	5×10^{-5}	++	++	5×10^{-4}	+++	+++	10^{-4}	P	P	5×10^{-5}	0	0

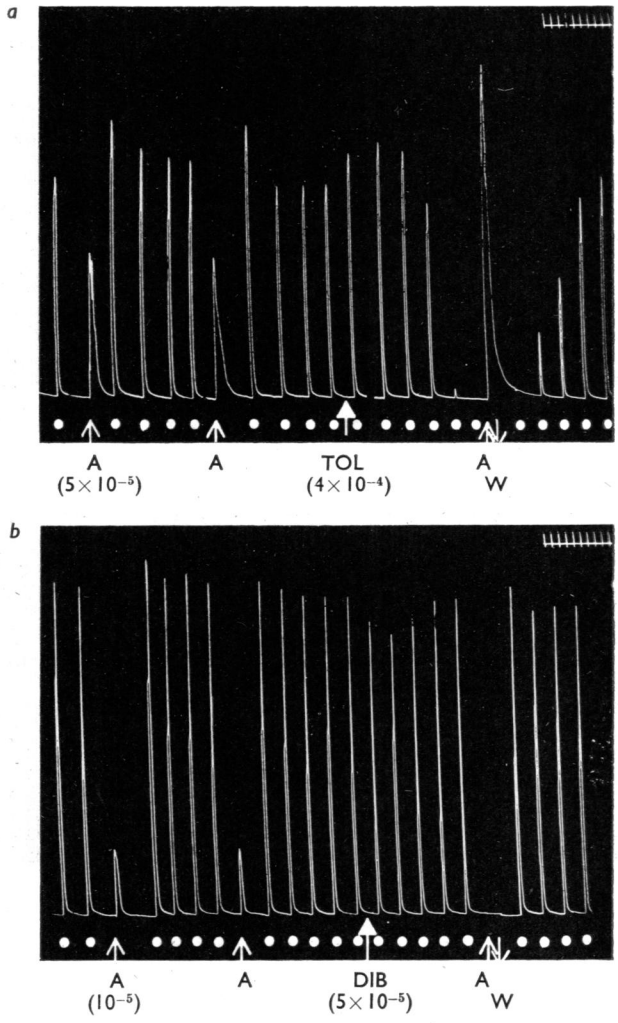

Fig. 6. Actions of (a) tolazoline (TOL) and (b) phenoxybenzamine (DIB) on the responses of the isolated guinea-pig hypogastric nerve-vas deferens preparation to added acetylcholine (A) and to nerve stimulation (○). Bath concentrations in g/ml. Time marker, 1 min intervals.

Guinea-pig pelvic nerve-rectum preparation. In concentrations sufficient to block the response to adrenergic nerve stimulation, each of the adrenergic blocking agents reduced the size of the responses of the guinea-pig rectum both to stimulation of the cholinergic pelvic nerve and to added acetylcholine (Table 1B). Piperoxan, yohimbine, phentolamine and phenoxybenzamine caused complete or almost complete block (Fig. 8). Bretylium and tolazoline caused more than 50% block. A striking result is that phenoxybenzamine blocked both responses in 10 min at a concentration 1/50th of that needed to block adrenergic nerve stimulation (Fig. 8d). In concentrations 1/100th to 1/10th of those shown in Table 1, most of

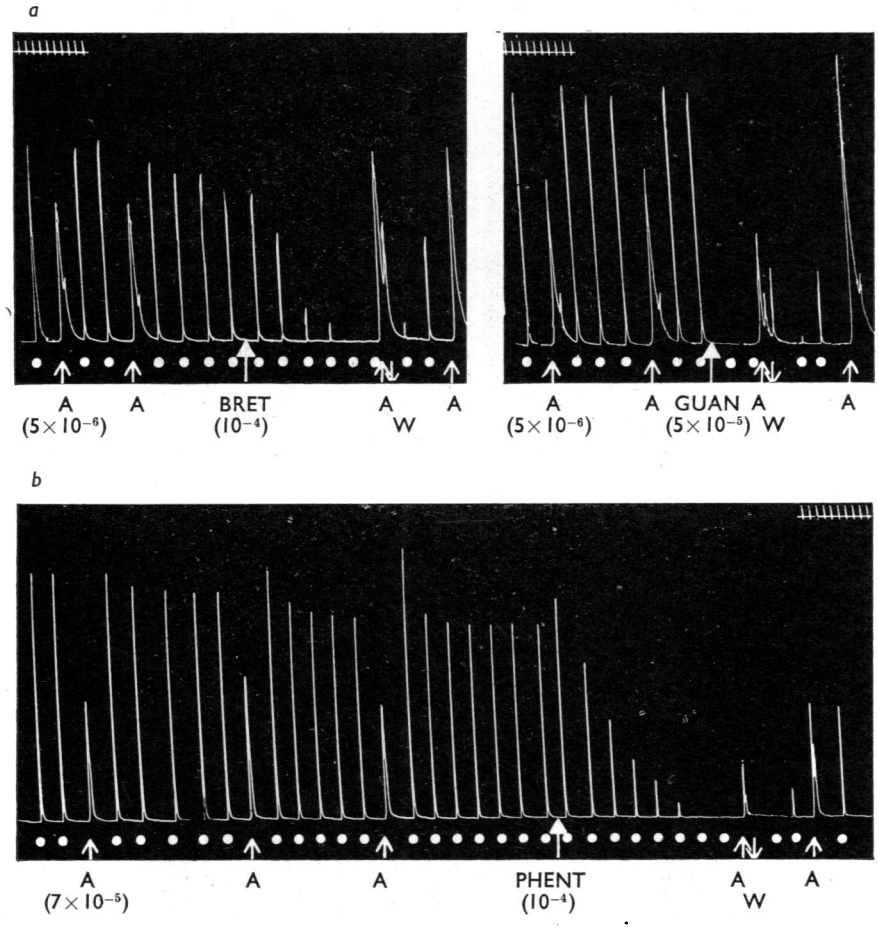

Fig. 7. Effects of (a) bretylium (BRET), (b) guanethidine (GUAN), and (c) phentolamine (PHENT) on the responses of the guinea-pig hypogastric nerve-vas deferens preparation to added acetylcholine (A) and to nerve stimulation (○). Bath concentrations in g/ml. Time marker, 1 min intervals.

the adrenergic blocking agents potentiated the nerve response rather than blocked it. This effect was particularly strong with yohimbine (Fig. 8c), tolazoline and piperoxan.

Toad "pelvic nerve"-bladder preparation. On this preparation, each of the adrenergic blocking drugs completely blocked the response to nerve stimulation. After block, the only drugs which did not always inhibit the response to acetylcholine by more than 50% were yohimbine, phentolamine and bretylium. The adrenergic blocking agents differed in the relative rates at which they blocked the response of the bladder to added acetylcholine and to nerve stimulation. Yohimbine (5×10^{-5} g/ml.) completely blocked the response of the toad bladder to nerve stimulation within 17 min, at which time the response to acetylcholine had been decreased by only 25% (Fig. 9a). In contrast, phenoxybenzamine (in the same concentration)

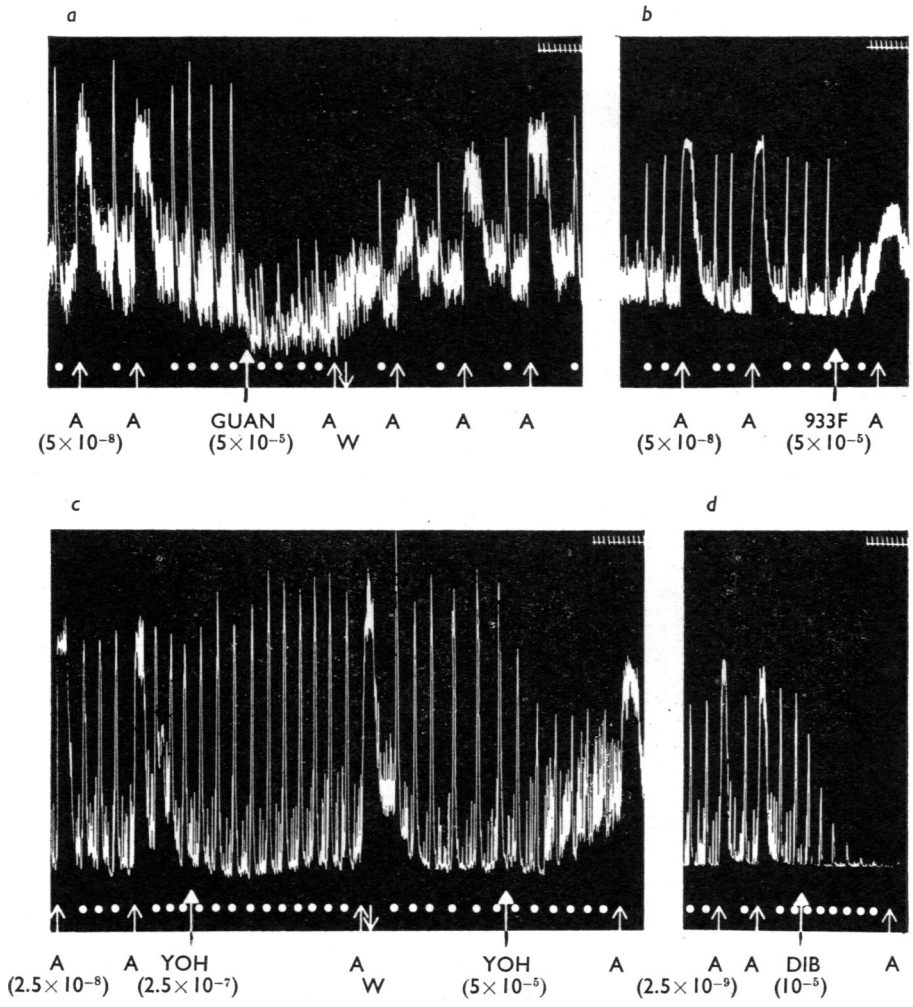

Fig. 8. Effect of (a) guanethidine (GUAN), (b) piperoxan (933F), (c) yohimbine (YOH) and (d) phenoxybenzamine (DIB), on the responses of the isolated guinea-pig pelvic nerve-rectum preparation to added acetylcholine (A) and to nerve stimulation (○). Bath concentrations in g/ml. Time marker, 1 min intervals.

required 200 min to block the response to nerve stimulation and less than 17 min to block completely the response to added acetylcholine (Fig. 9b). In no concentration did the adrenergic blocking agents potentiate the response to nerve stimulation.

Possum pelvic nerve-bladder preparation. In general the adrenergic blocking agents, like atropine, reduced the response to acetylcholine of this preparation more than the response to nerve stimulation. The greatest divergence of blocking rates was seen with guanethidine (10^{-4} g/ml.) which reduced the response to acetylcholine by 75% but had little or no effect on the response to nerve stimulation. No drug

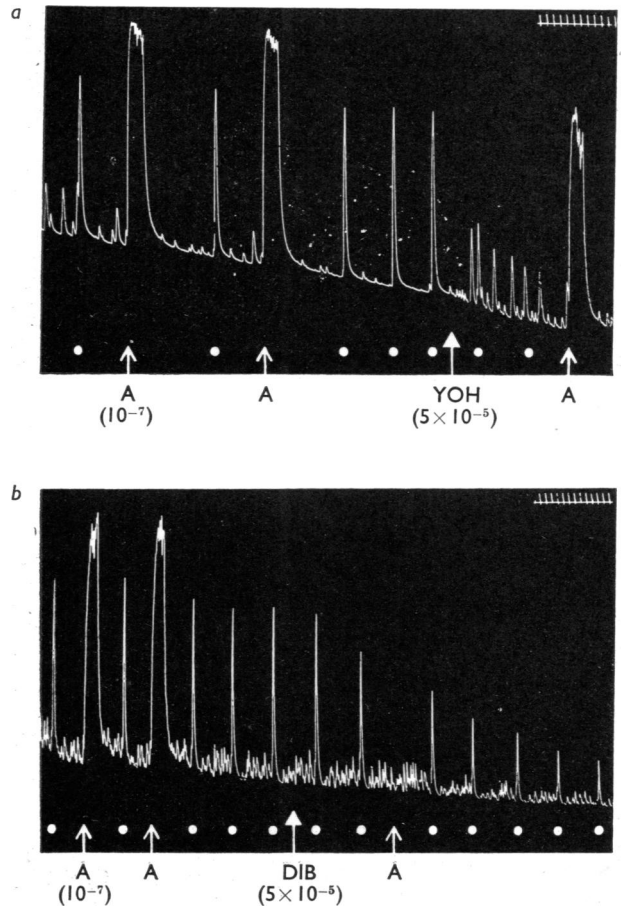

Fig. 9. Toad pelvic nerve-bladder preparation. Development of block to nerve stimulation (○) and to acetylcholine (A) after (a) yohimbine (YOH) and (b) phenoxybenzamine (DIB). Bath concentrations in g/ml. Time marker, 1 min intervals.

caused complete block of the response to nerve stimulation at the concentrations shown in Table 1. Tolazoline (5×10^{-4} g/ml.) potentiated the responses both to nerve stimulation and to acetylcholine, but at higher concentrations reduced both (Fig. 10a, b). Phenoxybenzamine (10^{-7} g/ml.) also caused potentiation, followed by a slight block.

The only general difference between the actions of the adrenergic blocking agents on adrenergically and cholinergically innervated preparations appeared to be that with the former there were often differences between the effects of the drugs on the responses to nerve stimulation and to added acetylcholine. With cholinergically innervated preparations the two responses were affected for the most part in parallel (Figs. 6 and 10).

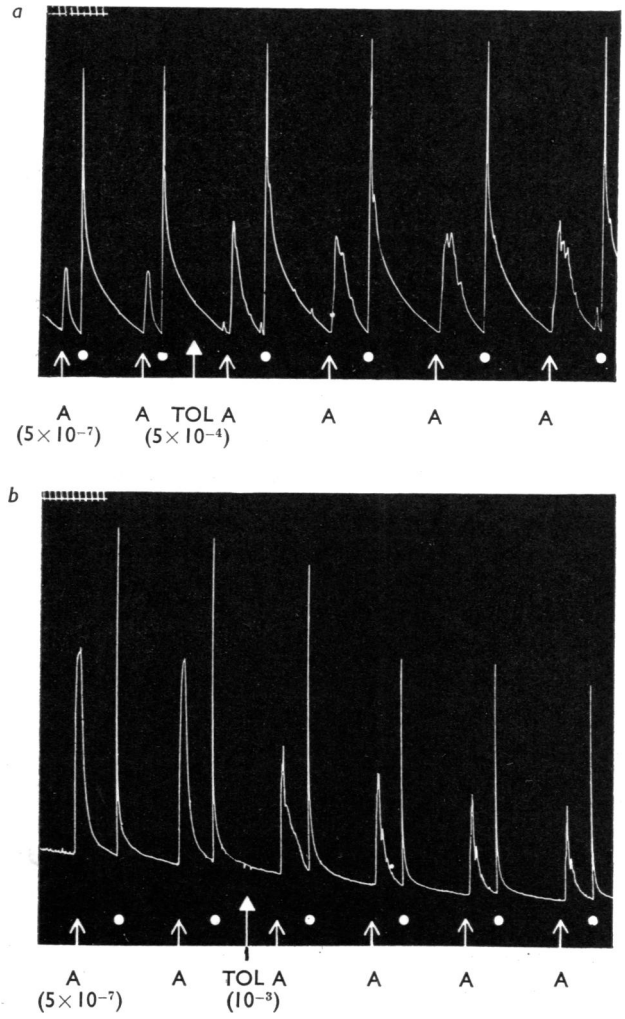

Fig. 10. Effects of two concentrations of tolazoline (TOL) on the responses of the isolated possum pelvic nerve-bladder preparation to added acetylcholine (A) and to nerve stimulation (○). Bath concentrations in g/ml. Time marker, 1 min intervals.

DISCUSSION

The results of these experiments emphasize the lack of specificity exhibited by the adrenergic blocking agents in concentrations necessary to block responses induced by adrenergic nerve stimulation. It appears that the block of cholinergic nerve-smooth muscle preparations by these agents is exerted on the muscle rather than on the nerve, for they can usually block a " muscarinic " action of acetylcholine relatively easily, although not as effectively as atropine. The mechanism of the acetylcholine blocking action of adrenergic blocking agents on the smooth muscle membrane is under investigation. In those cases where these substances block the

response to nerve stimulation more completely than the response to acetylcholine (for example, Fig. 9a), a presynaptic action cannot be excluded. For example, it has been suggested that yohimbine and bretylium have a local anaesthetic action (Nickerson, 1949 ; Boura & Green, 1959). Thus it can be seen that great care must be taken if adrenergic blocking agents are to be used to determine the nature of autonomic innervation. The use of autonomic drugs as analytical tools is not reliable unless combined with both histochemical examination of the preparation and assay of the substances released upon stimulation of the nerves.

A most striking result is that phenoxybenzamine, previously thought to block specifically α-adrenergic responses (Furchgott, 1954), is extremely potent against the excitatory actions of added acetylcholine and of cholinergic nerve stimulation ; moreover, it is often more effective against these than against adrenergic nerve stimulation.

In preparations sensitive to eserine, all the other agents, particularly tolazoline and piperoxan, could either potentiate or block cholinergic responses. It would seem from the results of experiments with dyflos on the guinea-pig rectum and on the toad bladder, which is insensitive to eserine, that this is due to an interplay between the anticholinesterase and acetylcholine blocking actions of the drugs. It is of interest to note that Dumont (1954) reported the absence of cholinesterase in the frog bladder. The anticholinesterase activity of some of these agents has been described by Boyd *et al.* (1960), who worked with the guinea-pig vas deferens preparation and found tolazoline to be the most potent drug, followed by piperoxan, yohimbine and phenoxybenzamine in that order. The activity of phentolamine, guanethidine and bretylium was not studied, but the results presented here suggest that they may each possess some anticholinesterase activity. Indeed, guanethidine appears to be quite a strong anticholinesterase since its potentiating action on cholinergic responses is abolished by dyflos. Only in preparations where the acetylcholine response is not potentiated by eserine can the antagonism of acetylcholine by drugs which possess anticholinesterase activity be seen unimpaired. Thus the dose/response curves for antagonism of acetylcholine by adrenergic blocking agents, obtained from the experiments on the toad bladder, probably give a true indication of the relative potencies of these drugs.

On the adrenergically innervated preparation, the effects of the drugs on the responses to nerve stimulation and to added acetylcholine were often in complete opposition ; the nervously mediated response was blocked whilst the response to added acetylcholine was potentiated. In contrast, on the cholinergically innervated preparations this was never the case. Usually, the nervously mediated response was affected similarly to the response to added acetylcholine although, on the possum and toad bladders, acetylcholine was sometimes more susceptible than the nerve response to the blocking actions of the anti-adrenergic agents. Resistance to atropine has been demonstrated in these two bladder preparations (Burnstock *et al.*, 1963 ; Burnstock & Campbell, 1963), and may explain this discrepancy. The resistance of nervously mediated responses to drug blockade has been frequently observed with both adrenergic and cholinergic nerve-smooth muscle preparations, and may be due, as Ursillo (1961) suggests, to differential penetration of barriers.

The validity of the criterion of adrenergic blocking activity, based on the concentrations of the drugs required to block responses to stimulation of the guinea-pig hypogastric nerve, may be open to question. Since the effects of hypogastric nerve stimulation are potentiated by anticholinesterases, higher concentrations may be needed to block completely the response to nerve stimulation than with an adrenergic nerve unaffected by eserine (if there is such a nerve). However, there is some debate as to the mechanism of transmission at all sympathetic nerve-smooth muscle junctions (see Burnstock & Holman, 1963), and there is no reason to believe that the hypogastric nerve-vas deferens preparation is atypical. The fact remains that it was not possible, with adrenergic blocking agents, to ascertain the nature of the motor innervation of any of the twelve preparations used whereas the action of atropine and of directly applied transmitter substances indicated that some, but not all, of the preparations were cholinergic.

A further objection to the criterion of adrenergic blocking activity chosen may be that anti-adrenaline agents are more effective against the directly applied transmitter substance than against that released upon nerve stimulation (Nickerson, 1949). The activities of some of these agents against added acetylcholine and added noradrenaline have been compared and the agents were found to be relatively specific for noradrenaline (Stone & Loew, 1952 ; Boyd *et al.*, 1960). However, our aim in these experiments has been to test the relative effectiveness of the adrenergic blocking agents against the transmitters released upon stimulation of adrenergic and cholinergic nerves, rather than against directly applied transmitter substances, and under these circumstances the agents appeared to be non-specific.

The implications in terms of mechanism of the results of this investigation are a matter for speculation. Both acetylcholine and adrenaline are capable of activating the same complex process which leads to depolarization of the smooth muscle membrane followed by contraction (Burnstock, 1960). Although the end result is the same, the receptor sites for these drugs are generally assumed to be distinct. There is some evidence suggesting this, for example atropine will block the response of a smooth muscle to acetylcholine in concentrations which do not affect the excitatory effects of catechol amines. However, as more becomes known of the actions of autonomic drugs, similarities rather than differences between the respective sites of action are emerging. Thus atropine blocks the excitatory actions of catechol amines in concentrations which do not appear to have a direct action on the muscle itself (Hildebrandt, 1920 ; Backman & Lundberg, 1922 ; Wehland, 1924 ; Regniers, 1926 ; Bussell, 1940 ; Burn & Dutta, 1948 ; Hamilton, 1960). Moreover, the present work confirms and extends previous findings of the acetylcholine blocking actions of adrenergic blocking agents (Ross, 1936 ; Boyd *et al.*, 1960 ; Kosterlitz & Lees, 1961). It may be suggested, therefore, that adrenergic and cholinergic blocking agents are not truly distinguishable. They could be considered to be members of a spectrum of blocking activity. Members at one end of the spectrum principally block cholinergic nerves, members at the other end principally adrenergic nerves. However, any member of this spectrum may, under certain conditions, show both adrenergic and cholinergic blocking activity. Thus atropine would have a strong affinity for acetylcholine receptors and only a weak affinity for noradrenaline

receptors, whilst phenoxybenzamine would appear to have a medium affinity for both. Similarly choline 2,6-xylyl ether bromide (xylocholine) has acetylcholine-like, anti-acetylcholine and anti-adrenergic properties (Willey, 1957).

Many adrenergic blocking agents are anticholinesterases (Boyd *et al.*, 1960), and atropine also appears to have this action (Ashford, Penn & Ross, 1962). It is of interest to note that eserine, a potent anticholinesterase, has atropine-like actions on the rabbit heart treated with dyflos (Quilliam & Strong, 1949) and is hence a member of the blocking spectrum. Whether this property indicates that cholinesterases are themselves the receptors, or whether it is an accidental feature of the structure necessary for occupation of acetylcholine receptors, is debatable. Another unexplained action of many adrenergic blocking agents is their potentiation of the actions of catechol amines (Jang, 1940). However, whatever the situation may be, it seems that there is a considerable chemical or positional overlap between adrenaline and acetylcholine receptor sites which is sufficient to make pharmacological differentiation difficult.

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