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POTENTIATION OF RESPONSES TO NORADRENALINE AND REVERSAL OF SYMPATHETIC NERVE BLOCKADE IN THE GUINEA-PIG VAS DEFERENS

BY

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The block of sympathetic nerves caused by guanethidine in various isolated organs may be reversed by a number of substances (Day, 1962; Bentley, 1962; Day & Rand, 1963; Bentley & Sabine, 1963). These antagonizing agents include sympathomimetic and parasympathomimetic drugs, histamine and 5-hydroxytryptamine. The mode of action of amphetamine has been investigated by Day & Rand (1963) who showed that this drug competes with guanethidine for absorption sites on sympathetic nerves. They found, however, that noradrenaline does not show this competitive antagonism. Matsumoto & Horita (1962) have suggested that the ability of various sympathomimetic amines to antagonize the effects of bretylium on the nictitating membrane involved potentiation of the diminished amounts of transmitter released from the sympathetic nerve. This hypothesis was investigated in more detail in the present study using guinea-pig isolated vas deferens preparations. The ability of various substances to potentiate the effects of noradrenaline was compared with their action in reversing the sympathetic block caused by guanethidine, low calcium, procaine and previous treatment with reserpine.

METHODS

Guinea-pigs weighing 500 to 750 g were stunned and bled. Both vasa deferentia were removed, stripped of their mesenteric investments, and set up in 10-ml. organ-baths at 34° C in the solution described by Holman (1958). They were gassed with 95% oxygen and 5% carbon dioxide. Responses were recorded with frontal-writing levers on a smoked drum.

Sympathetic block and its reversal

The motor fibres to the vas deferens in the hypogastric nerve are believed to relay in a ganglion close to its entry into that organ (Ferry, 1962; Sjöstrand, 1962; Bentley & Sabine, 1963; Kuriyama, 1963). For this reason most experiments were done on the stripped vas deferens, stimulated transmurally using a platinum electrode inserted in the lumen (Bentley & Sabine, 1963), while a few other experiments were done for comparison with the hypogastric nerve-vas deferens preparation. Paired preparations were set up in organ-baths of 80 ml. capacity. A Grass model S4-D stimulator, delivering pulses of 1 msec duration at a frequency of 50 shocks/sec for periods of 5 sec each 2 min was used. Conditions were arranged so that maximal and submaximal responses alternated. This was achieved either by altering the voltage between each response, or by alternating the stimulus frequency between 50 and 20 shocks/sec.

When the control responses were steady, the blocking drug was added to the first preparation at the same time as the antagonizing drug was added to the other. Stimulation was continued until the responses were

steady again in both preparations. Then the antagonizing drug was added to the first preparation, and the blocking drug to the second. Stimulation was continued until no further change was seen in either preparation. Both baths were then washed out, and stimulation was continued for a further 10 to 15 min.

In preparations from reserpine-treated guinea-pigs, only the ability of various substances to augment electrically induced contractions was studied.

The potentiation of responses to noradrenaline

Responses to noradrenaline were obtained from stripped vas deferens preparations, using doses which gave neither near-maximal nor just detectable responses. Two dose levels were used, one producing approximately twice the contraction of the other. These were applied in random order until at least four consecutive responses were consistent. The noradrenaline was added to the bath at exactly 5-min intervals, and was left in contact with the tissue for 30 sec, by which time the contraction had reached a maximum. When the control responses were steady the vas deferens was treated with either guanethidine, procaine or low calcium (0.25 mM). Responses to noradrenaline were again tested until they were reproducible. Finally the vas deferens was treated with a combination of the sympathetic blocking agent plus antagonist and the responses to noradrenaline were tested a third time. When changes in sensitivity to noradrenaline occurred the doses were adjusted until the responses matched the previous contraction.

Some experiments were also carried out using vasa deferentia taken from guinea-pigs that had previously been treated with reserpine, 1 mg/kg/day injected intraperitoneally for 2 to 3 days.

Drugs

All drugs were freshly diluted each day in 0.9% saline. Carbachol (B.D.H.), 3,4-dihydroxyphenylethylamine (dopamine, Nutritional Biochemicals Corp.), procaine (Bull Labs.), acetyl-β-methylcholine (methacholine, Light) and tyramine (Calbiochemicals) were used as hydrochlorides, atropine (T. & H. Smith), (±)-amphetamine (Light), guanethidine (Ciba) and physostigmine (T. & H. Smith) as sulphates, arecoline (B.D.H.) as hydrobromide, (−)-metaraminol (Merck Sharpe & Dohme) and (−)-noradrenaline (Sterling) as bitartrates, histamine (B.D.H.) as the acid phosphate, neostigmine (Light) as the methylsulphate, and 5-hydroxytryptamine (May & Baker) as the creatine sulphate. Lyophilized angiotension (Hypertensin, Ciba) and reserpine (Serpasil, Ciba) were used from ampoules.

All drug concentrations are expressed as the salts.

RESULTS

Blockade of sympathetic nerve and its reversal by various agents

The substances tested for ability to reverse the block due to guanethidine showed various effects on the response of the untreated vas deferens to electrical stimulation. In all experiments both maximal and submaximal responses were increased. The average percentage increases in the maximal response were as follows (number of experiments in parentheses): carbachol 1×10^{-7}, 29% (3) and 1×10^{-6}, 47% (6); noradrenaline 1×10^{-6}, 28% (4) and 2×10^{-6}, 56% (3); amphetamine 1×10^{-6}, 8% (3) and 2×10^{-6}, 13% (4); tyramine 1×10^{-5}, 20% (8); 15 mM-potassium, 26% (4); histamine 2×10^{-6}, 7% (4); 5-hydroxytryptamine 2×10^{-6}, 3% (5); angiotension 2×10^{-6}, 12% (3); and neostigmine 1×10^{-6}, 12% (3). In most instances the submaximal responses were increased by the above drugs to a greater extent than the maximal contractions.

Guanethidine (5×10^{-6}), procaine (1×10^{-4}) and lowering the calcium level to 0.25 mM all caused reductions in the response of the vas deferens to electrical stimulation. Usually, the effect was more marked when the stimulus was applied to the hypogastric nerve than when transmural stimuli were used. Procaine and low calcium often caused complete block, and never less than 70% reduction, while guanethidine was less consistent on the transmurally stimulated preparations, seldom giving full block, and occasionally causing

reductions of only 30%. With guanethidine, submaximal responses were always depressed to a greater degree than were maximal responses, irrespective of whether they were produced by lowering the voltage or the frequency of the stimulus. The extent of the block due to reserpine was impossible to estimate accurately, since no controls were available from the same animals before treatment. However, in most instances, the contractions produced by preparations taken from reserpinized animals were smaller than normal, and progressively diminished as the experiment continued.

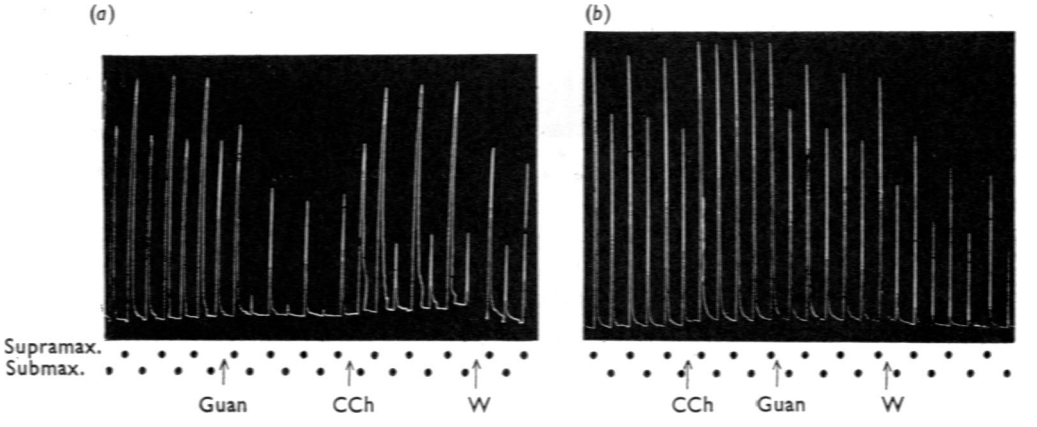

Fig. 1. Responses of guinea-pig vas deferens preparations, stimulated transmurally with alternating supramaximal and submaximal voltages. (*a*) At Guan, guanethidine (5×10^{-6}) and at CCh, carbachol (10^{-6}) were added to the bath, without washing out the guanethidine. W, Wash out. (*b*) At CCh, carbachol (10^{-6}) and at Guan, guanethidine (5×10^{-6}) were added to the bath, without washing out the carbachol. The blocking action of guanethidine was slight while carbachol was present, but appeared after both drugs had been washed out.

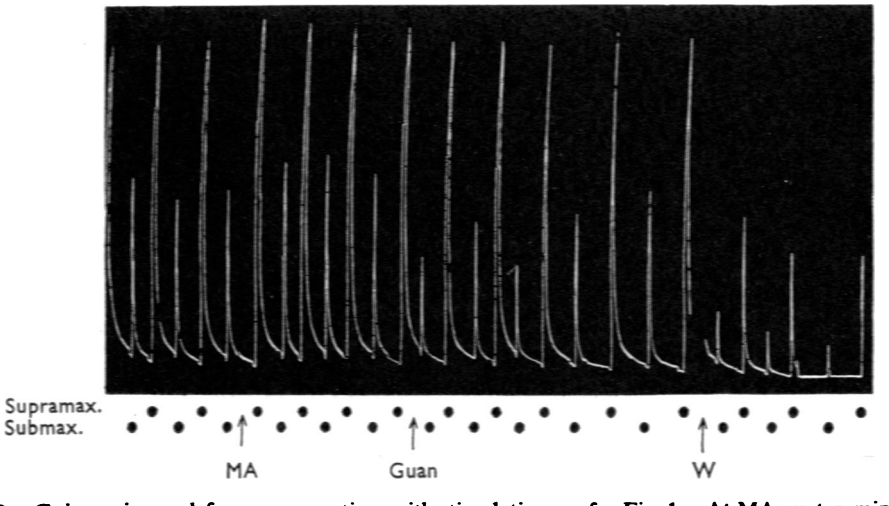

Fig. 2. Guinea-pig vas deferens preparation, with stimulations as for Fig. 1. At MA, metaraminol (10^{-6}) and at Guan, guanethidine (5×10^{-6}) were added. W, Wash out. Metaraminol protected against block by guanethidine, but the block appeared after both drugs had been washed out.

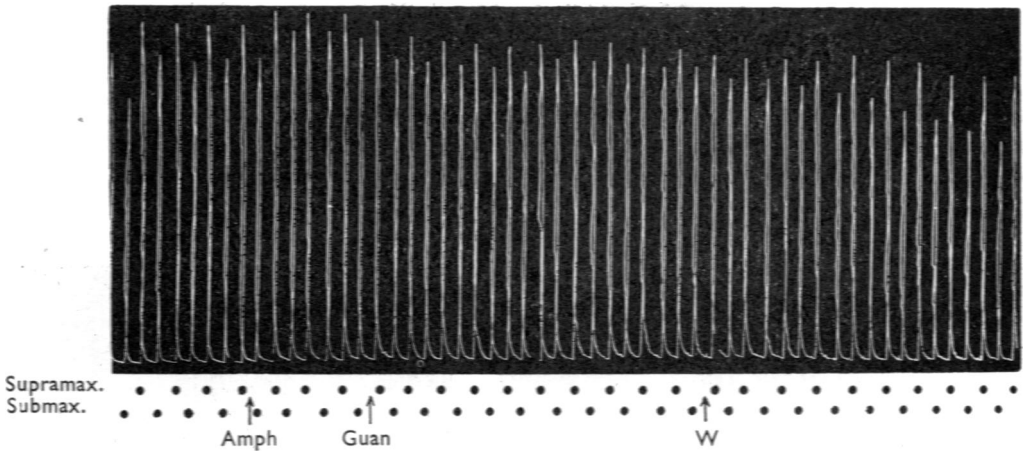

Fig. 3. Guinea-pig vas deferens preparation, with stimulations as for Fig. 1. At Amph, amphetamine (10^{-6}) and at Guan, guanethidine (5×10^{-6}) were added. W, Wash out. Guanethidine produced no significant block in the presence of amphetamine, and no block appeared after washing out the bath.

TABLE 1

ABILITY OF VARIOUS AGENTS TO PROTECT AGAINST A GUANETHIDINE-INDUCED BLOCK OF THE ELECTRICALLY STIMULATED GUINEA-PIG VAS DEFERENS

Pairs of numbers give percentage block for guanethidine with interacting substance and percentage block with guanethidine only. * Hypogastric nerve-vas deferens preparations

Interacting substance	Concentration	Block (%) for guanethidine with and without interacting substance			
Arecoline	2×10^{-6}	40/94*	26/90*	32/84	4/79
Methacholine	2×10^{-6}	14/81*	25/75	36/56	
Carbachol	10^{-7}	53/78	32/89	77/80	
	10^{-6}	47/100*	42/100*	40/100*	40/94*
		20/80	30/60	4/61	
Neostigmine	10^{-6}	34/100*	63/82*	32/82*	
		41/94	46/84	76/90	69/70
Physostigmine	10^{-6}	35/56	53/80	29/47	
Potassium	15 mM	27/100*	60/100*	36/59	56/93
Noradrenaline	10^{-6}	0/62	27/50	22/69	
	2×10^{-6}	18/100*	19/82*		
Tyramine	10^{-5}	17/60*	19/100*	63/94*	
		63/94	19/100	14/77	9/43
Amphetamine	10^{-6}	25/92*	0/100	21/56	
Dopamine	5×10^{-6}	100/100*	15/73	52/71	
Histamine	5×10^{-6}	24/59*	67/100*	72/77	72/96
5-Hydroxytryptamine	2×10^{-6}	29/65	71/61	82/52	
	5×10^{-6}	89/69			
	10^{-5}	59/43*	46/39*	27/18	
Angiotensin	$2{\cdot}5 \times 10^{-6}$	64/100*	65/100*		
	5×10^{-6}	30/88	51/77	88/68	
Metaraminol	10^{-6}	26/56	9/46	25/62	5/27

Guanethidine. Several substances when added before guanethidine were effective in reducing its blocking action. An exact comparison of their ability to antagonize the block is impossible, since the degree of protection each drug afforded varied from one experiment

to another. The substances that consistently gave marked protection were arecoline 2×10^{-6}, methacholine 2×10^{-6}, carbachol 1 to 10×10^{-7} (Fig. 1), neostigmine 1×10^{-6}, physostigmine 1×10^{-6}, 15 mM-potassium, noradrenaline 1×10^{-6}, tyramine 1×10^{-5}, amphetamine 1×10^{-6} (Fig. 3) and metaraminol 1×10^{-6} (Fig. 2). Dopamine at levels of 4×10^{-6} or lower gave very inconsistent results, but was moderately effective at 5×10^{-6}. Similarly, histamine was ineffective at 2×10^{-6}, but had some action at 5×10^{-6}. 5-Hydroxytryptamine at doses between 2×10^{-6} and 1×10^{-5} almost always increased the blocking action of guanethidine, though, rather strangely, if added after the block developed it always produced some small degree of recovery. Angiotension at 5×10^{-6} gave some protection in two out of three experiments (Table 1).

The ability of the different substances to reverse an existing block varied considerably. After the addition of either tyramine or amphetamine, recovery of responses was slow, seldom being complete in less than 15 min. All the other substances, however, produced their effect immediately and in some instances (particularly with histamine) there was subsequently some degree of fall off. When the bath was washed out, without replacing guanethidine, failure of responses rapidly occurred in all instances except with amphetamine. It is difficult to give an exact comparison of the relative potencies of these substances in reversing the effects of guanethidine, since there is so great a variation from one experiment to another in both the degree of block caused by guanethidine, and the extent to which this was reversed by the interacting substances. The following substances have been observed to reverse completely the effect of guanethidine: arecoline 2×10^{-6}, carbachol 1×10^{-6}, methacholine 2×10^{-6}, noradrenaline 1×10^{-6}, dopamine 5×10^{-6}, metaraminol 1×10^{-6}, amphetamine 1×10^{-6}, tyramine 1×10^{-5} and 15 mM-potassium. Noradrenaline and carbachol were most consistent in producing a full reversal. Carbachol

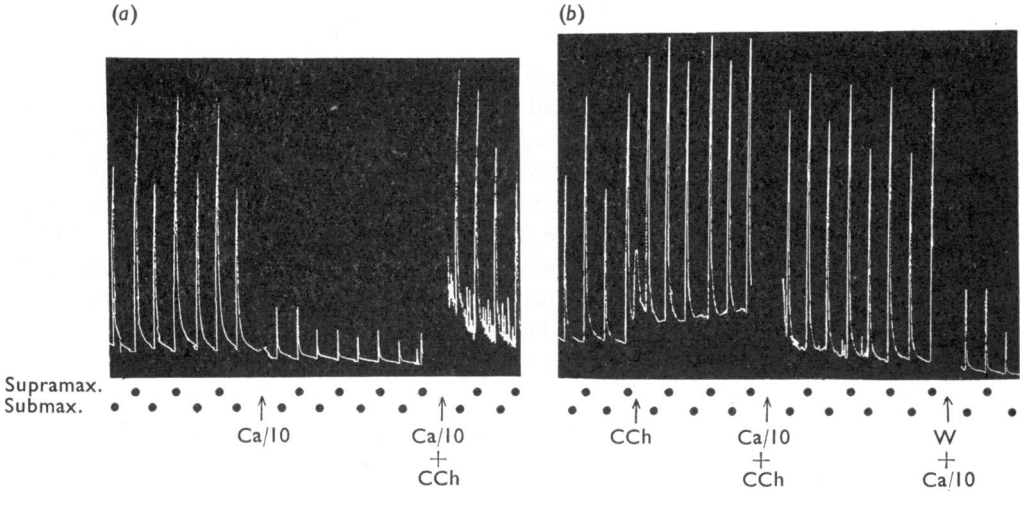

Fig. 4. Guinea-pig vas deferens preparations, with stimulations as in Fig. 1. (*a*) At Ca/10, the preparation was placed in Holman solution containing 0.25 mM-calcium; at CCh, carbachol (2×10^{-6}) was added with the same calcium concentration. (*b*) At CCh, carbachol (2×10^{-6}) and at Ca/10+CCh, 0.25 mM-calcium in Holman solution with carbachol; at W+Ca/10, the bath was washed out with low calcium solution, but without carbachol. Carbachol protected against and reversed the block due to low calcium.

at 1×10^{-7} caused some degree of reversal, and this increased when the concentration was raised to 5×10^{-7}, but complete recovery was not seen at levels below 1×10^{-6}. 5-Hydroxytryptamine, 2×10^{-6} to 1×10^{-5}, always gave some reversal, but the extent was not related to the dose level. The other substances were all considerably weaker in their action.

Low calcium. When added before treatment with 0.25 mM-calcium, the following substances gave various degrees of protection against block of the electrically induced responses of the vas deferens: carbachol 1×10^{-6} (Fig. 4), noradrenaline 2×10^{-6}, tyramine 1×10^{-5} and amphetamine 1×10^{-6}. Carbachol was the most effective. 15 mM-Potassium, histamine 2×10^{-6} and 5-hydroxytryptamine 2×10^{-6} were ineffective (Table 2).

TABLE 2

ABILITY OF VARIOUS AGENTS TO PROTECT AGAINST DEPRESSION BY LOW CALCIUM OF THE ELECTRICALLY INDUCED RESPONSE OF THE VAS DEFERENS

Pairs of numbers give percentage block for 0·25-mM calcium with interacting substance and percentage block with 0·25 mM-calcium only. * Hypogastric nerve-vas deferens preparations

Interacting substance	Concentration	Block (%) for low calcium with and without interacting substance			
Carbachol	10^{-6}	19/96	2/85	44/95*	35/100*
Noradrenaline	2×10^{-6}	48/84	65/88	88/100*	83/85*
Tyramine	10^{-5}	56/83	74/100	84/87	
Amphetamine	10^{-6}	30/72	59/100	69/100*	47/94*
Potassium	15 mM	92/93	73/79	78/100	84/82
5-Hydroxytryptamine	2×10^{-6}	90/86	84/76		
Histamine	2×10^{-6}	55/57	52/93	97/84	

When added after the depression of responses by low calcium had occurred, only carbachol and noradrenaline had any marked ability to reverse the block, but even these two substances were not completely effective. Tyramine, histamine, amphetamine and 5-hydroxytryptamine showed weak and variable activity, while 15 mM-potassium increased the blocking action of low calcium (Sabine & Bentley, unpublished).

Procaine. Of the six substances tested for ability to protect against procaine, only 15 mM-potassium and noradrenaline showed any marked activity. Amphetamine, 1×10^{-6}, appeared to potentiate the block, while tyramine, 1×10^{-5}, and histamine, 5×10^{-6}, were without effect. Carbachol, 1×10^{-6}, was inactive, and at 4×10^{-6} it gave some protection in only one of three experiments (Table 3). However, when added after the block by procaine had occurred, it caused some recovery of responses, but was less effective than either 15 mM-potassium or noradrenaline. The action of these latter two drugs when added after procaine was variable, but both on occasions produced complete recovery. Histamine and tyramine were much weaker, and amphetamine was inactive.

Reserpinized preparations. Preparations taken from animals that had previously been treated with reserpine showed wide variations in their responses to added drugs. In general, those preparations which gave small contractions were potentiated to the greater extent by added drugs. Several times it was noted that, as the control contractions diminished throughout the course of an experiment, a larger percentage potentiation was obtained by a second application of a drug. Carbachol 1×10^{-6} (Fig. 5), noradrenaline 1×10^{-6} and histamine 2×10^{-6} were most active in increasing the size of contractions. The contractions were increased from 1.1- to 7-times in the presence of histamine (mean of six experiments=2.5-times), in carbachol from 1.2- to 4.7-times (mean of twelve experi-

TABLE 3

ABILITY OF VARIOUS AGENTS TO PROTECT AGAINST PROCAINE-INDUCED DEPRESSION OF THE ELECTRICALLY INDUCED RESPONSE OF THE VAS DEFERENS

Pairs of numbers give percentage of block for procaine (10^{-4}) with interacting substance and percentage block for procaine only. * Hypogastric nerve-vas deferens preparations

Interacting substance	Concentration	Block (%) for procaine with and without interacting substance			
Carbachol	10^{-6}	100/75*	57/47	92/84	
	4×10^{-6}	20/80	67/47	89/82	
Noradrenaline	2×10^{-6}	100/42*	100/100*	15/60	26/53
Tyramine	10^{-5}	60/80	53/50	66/48	
Amphetamine	10^{-6}	90/74	100/36	77/72	
Potassium	15 mM	24/100	11/75	17/100	
Histamine	2×10^{-6}	78/58	63/55	53/85	

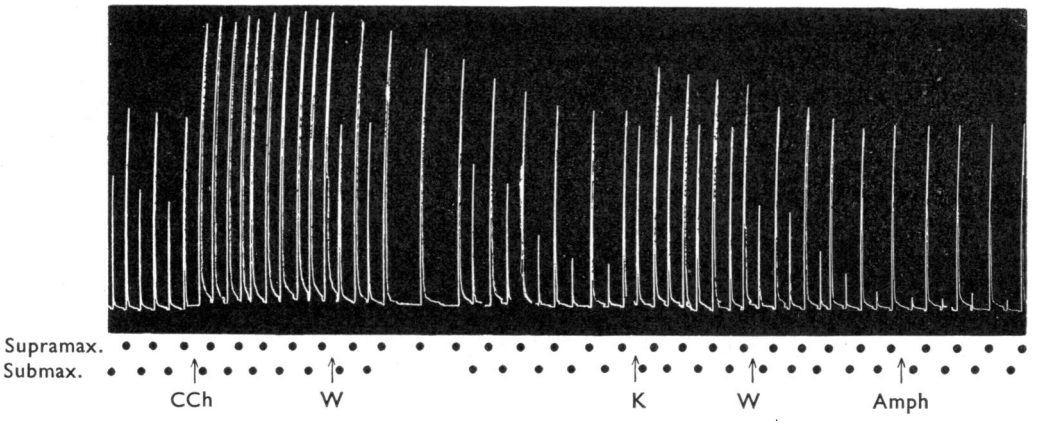

Fig. 5. Guinea-pig vas deferens preparation from a reserpinized animal, with stimulations as in Fig. 1. At CCh, carbachol (10^{-6}) was added. At K, Holman solution containing 15 mM-potassium. At Amph, amphetamine (10^{-6}). W, Wash out. Carbachol produced a considerable potentiation of the responses, raised potassium greatly potentiated the submaximal responses, but had little effect on the maximal ones, while amphetamine had no detectable effect.

TABLE 4

THE EFFECT OF VARIOUS SUBSTANCES ON THE RESPONSES TO ELECTRICAL STIMULATION OF VAS DEFERENS PREPARATIONS FROM RESERPINIZED GUINEA-PIGS

Figures in parentheses indicate numbers of experiments

Drug	Concentration	Increase (%) in contraction height	
		Range	Mean
Carbachol	5×10^{-7}	50, 136	93 (2)
	10^{-6}	12–473	183 (12)
Noradrenaline	5×10^{-7}	40	— (1)
	10^{-6}	5–525	136 (12)
Histamine	2×10^{-6}	10–700	249 (6)
5-Hydroxytryptamine	2×10^{-6}	16–153	65 (6)
Angiotensin	2×10^{-6}	0–129	46 (7)
Potassium	15 mM	4–100	37 (6)
Amphetamine	10^{-6}	0–100	30 (4)
Tyramine	10^{-5}	0– 26	5 (6)

ments=1.8-times) and in noradrenaline from 1.05- to 5.25-times (mean of twelve experiments=1.4-times). The other substances tested all had much weaker actions (Table 4).

Interactions with atropine. The possibility existed that the effects of carbachol were due to a release of catechol amines from tissue stores by a nicotinic action, though the high activity of arecoline and methacholine argued against this. Nevertheless, this suggestion was tested in the following manner. Atropine, 1×10^{-6}, was added to one of a pair of vas deferens preparations. This caused a small reduction in the responses to electrical stimulation. Carbachol, 1×10^{-6}, was then added to both preparations, and then guanethidine, 5×10^{-6}. It was found that, in the presence of atropine, carbachol did not protect from block due to guanethidine. Further, when atropine was added to the second preparation which had already been treated with guanethidine plus carbachol, an immediate and marked inhibition of the responses occurred. However, atropine did not affect the ability of amphetamine or noradrenaline to protect the preparation from block by guanethidine, or to reverse an existing depression. These results agree with those of Sjöstrand (1961), who showed that the potentiating action of acetylcholine on the hypogastric nerve-vas deferens preparation was abolished by atropine, but the effects of adrenaline were not altered.

Similarly, atropine abolished the ability of carbachol to reverse the effects of low calcium.

The response of the isolated vas deferens to noradrenaline after treatment with various substances

It was not always possible to estimate the degree of potentiation with high accuracy as the slope of the dose/response curve occasionally altered in the presence of the various drugs. Also in 15 mM-potassium plus procaine, the vas deferens sometimes beat spontaneously, obscuring the responses to noradrenaline.

Effects of guanethidine and its antagonists. Guanethidine in concentrations of 5×10^{-6} in the bath markedly potentiated the effects of noradrenaline. Increases in sensitivity of

TABLE 5

CHANGE IN SENSITIVITY OF GUINEA-PIG ISOLATED VAS DEFERENS PREPARATION TO NORADRENALINE WHEN INTERACTING SUBSTANCES WERE ADDED TO TISSUES ALREADY TREATED WITH GUANETHIDINE (5×10^{-6}), PROCAINE (10^{-4}) OR 0·25 mM-CALCIUM

Numbers give proportionate change in sensitivity to noradrenaline (1=no change in sensitivity); ↓, sensitivity reduced to below level before antagonist added; ↑ ↓, brief increase in sensitivity, then failure; †, slight increase; ↑ +, potentiation+beating. Values for the experiments without added antagonist of sympathetic block are means and standard errors

Antagonist of sympathetic block	Change in response to noradrenaline for tissue treated with		
	Guanethidine	Procaine	Low calcium
None	17·6±2·47	2·9±0·6	Great reduction
Carbachol (1 to 2×10^{-6})	↓, ↓, 1, 1·8, 2·5	↓, ↓, 1, 1, 1, 1, 1·5	↓, ↓, ↓, 1, 1, 1·5
Neostigmine (10^{-6})	1·8, 2		
Amphetamine (10^{-6})		2, 3, 3	↑ ↓, 1, 1, 1
Dopamine (5×10^{-6})	1·5, 2, 20		
Tyramine (10^{-5})	↓, ↓, ↓, 1·2, 2	↓, 1·5, 1·5, 2, 2, 2–3, 4	↑ ↓, 1, 1, †
Metaraminol (10^{-6})	2, 2		
Histamine (1 to 5×10^{-6})	1, 1, 1, 2, 2, 2, 2·5, 4		↑ ↓, ↑ ↓
5-Hydroxytryptamine (1 to 5×10^{-6})	2, 3, 1, 1		↑ ↓, ↑ ↓
Angiotensin (2×10^{-6})	1, 1·2, 2		
Potassium (15 mM)	↓, 1, 1·5, 2	↑ +, ↑ +, ↑ +, 1·5, 2·5, 6, 7, 7·5	↓, 1

2- to 50-times have been seen, though usually this varied between 10- and 20-times (mean of twenty-four experiments, 17.6±2.47). The agents that antagonized the sympathetic nerve block of guanethidine also potentiated noradrenaline in various degrees, amphetamine and 15 mM-potassium being most effective, while neostigmine was least active. However, when added to preparations that had already been treated with guanethidine, the guanethidine antagonists caused little or no further increases in sensitivity to noradrenaline and, in some instances, the existing potentiation was actually reduced. These results are summarized in Table 5.

Effects of procaine and other substances. Procaine, 1×10^{-4}, usually caused potentiation of noradrenaline up to fourfold, while in a few instances a small reduction in sensitivity was seen (mean of nineteen experiments, 2.9±0.6). Tyramine and amphetamine when added after procaine caused only small further increases in sensitivity, from 2- to 3-times that seen with procaine alone. Carbachol was even less effective in potentiating noradrenaline in the presence of procaine, and in two experiments actually reduced the sensitivity. Potassium, 15 mM, was the only treatment that caused any further significant potentiation, though this varied from 1.5- to 7.5-times (Table 5). Occurrence of spontaneous beat in some preparations after treatment with 15 mM-potassium and procaine precluded any estimation of the degree of potentiation, but a clear increase was always seen.

Effects of low calcium and other substances. In low calcium solutions (0.25 mM) the responses to noradrenaline were severely depressed. Carbachol and amphetamine showed no ability to reverse this depression, while 5-hydroxytryptamine gave only a temporary recovery, followed rapidly by a complete block. Tyramine in one experiment reversed the depression, but in two others increased it (Table 5).

DISCUSSION

The purpose of the experiments described above was to investigate the mechanisms by which various substances antagonized the sympathetic blockade caused by guanethidine. A comparison was made of the ability of the antagonizing substances to potentiate added noradrenaline in the presence of guanethidine (which itself strongly potentiates noradrenaline) with their ability to prevent or reverse the block of sympathetic nerves caused by guanethidine. The object was to test the theory that after partial or complete sympathetic nerve block there was still some release of transmitter in reduced amounts. In the presence of substances that potentiated noradrenaline, this reduced amount of transmitter might become more effective. For comparison, three other manœuvres were employed to reduce sympathetic transmission. These were lowered calcium, which is known to reduce the output of transmitter at the somatic neuromuscular junction (Fatt & Katz, 1952) and which reduces the inhibitory effect of sympathetic nerve stimulation in the rabbit isolated ileum (Burn & Gibbons, 1964); reserpine, which depletes the stores of noradrenaline in tissues (Burn & Rand, 1957), and procaine, which depresses conduction in nervous tissue.

In comparing the actions of drugs on responses mediated by stimulation of sympathetic nerves with those produced by the addition of noradrenaline, two assumptions are necessary. The first is that noradrenaline is in fact the sympathetic transmitter in the vas deferens. The second is that the smooth muscle receptors responding to added noradrenaline are the

TABLE 6

COMPARISON OF THE ABILITY OF VARIOUS SUBSTANCES TO POTENTIATE NORADRENALINE ON THE ISOLATED VAS DEFERENS, AND TO ANTAGONIZE SYMPATHETIC NERVE BLOCK PRODUCED BY GUANETHIDINE, PROCAINE, RESERPINE OR LOW CALCIUM

+++, Great, sometimes complete antagonism of nerve block, great potentiation of noradrenaline, or of responses to electrical stimulation; ++ moderate, never complete antagonism of nerve block, moderate potentiation of other effects; +, weak action; ±, weak, variable action; —, no change; 0, increase of depressant action

Substance	Potentiation on untreated preparation	Interaction with guanethidine: Further potentiation	Interaction with guanethidine: Antagonism of nerve block	Interaction with procaine: Further potentiation	Interaction with procaine: Antagonism of nerve block	Interaction with low calcium: Potentiation	Interaction with low calcium: Antagonism of nerve block	Interaction with reserpine: potentiation of electrically induced contraction
Carbachol	++	±	+++	+	±	0	+++	+++
Tyramine	++	—	+++	+	±	0	+	0
Amphetamine	+++	—	+++	+	0	0	++	0
15 mM-Potassium	++	±	++	++	+++		—	±
Histamine	++	±	±		±		0	+++
5-Hydroxytryptamine	+	+	0			±	±	+
Noradrenaline			+++		++		++	+++

same as those activated by transmitter released from the sympathetic nerve, or, if not actually the same, at least they can act as models for the presumably less accessible receptors beneath the nerve endings. Burnstock & Holman (1961), using electrophysiological techniques, have concluded that the junction potentials seen in guinea-pig hypogastric nerve-vas deferens preparations were probably produced by noradrenaline, and hence the first assumption seems reasonable. There are reasons, however, for believing that the receptors activated by added noradrenaline are not the same as those activated by transmitter from the sympathetic nerve. Jowett & Holman (1964) have shown that the adrenolytic drug phentolamine blocks the action of added noradrenaline on the guinea-pig vas deferens at concentrations which do not block the response to nerve stimulation. Furthermore, I have found that guanethidine can markedly potentiate added noradrenaline within 1 min of its addition to a vas deferens. At this time, no effect can be detected on the response to electrical stimulation. This very rapid sensitizing action of guanethidine is similar to the effect of cocaine on the cat nictitating membrane that Fleming & Trendelenburg (1961) noted. Nevertheless there seems to be no reason to assume that all these adrenotropic α-receptors in the vas deferens do not behave identically, irrespective of their location within the tissue.

It has been shown in this paper that a number of substances can antagonize the sympathetic nerve blocking action of guanethidine on the isolated vas deferens. These include both directly and indirectly acting sympathomimetic amines, parasympathomimetic agents, and high potassium. Histamine and 5-hydroxytryptamine have a weaker action. In contrast, the nerve block produced by low calcium (0.25 mM), by procaine, and by treatment with reserpine *in vivo*, is antagonized by only a few of the above agents. The effects of procaine are most effectively reversed by high potassium, while the other substances are much weaker or ineffective. The depression caused by low calcium, on the other hand, is reversed convincingly only by carbachol, while the sympathetic amines are much less effective. The small contractions of reserpinized tissues are augmented by carbachol, histamine and noradrenaline, but not by indirectly acting sympathomimetic agents, or by high potassium.

It has also been shown that most of the antagonists of guanethidine, like guanethidine itself, can potentiate the effects of noradrenaline added to the isolated vas deferens, but, when added after guanethidine has produced its effect, none of these substances cause any further significant potentiation of noradrenaline, while some actually reduce the tissue's sensitivity. Similarly, none of the substances tested showed any ability to increase the reduced responses to noradrenaline in 0.25 mM-calcium. In procaine, only 15 mM-potassium markedly increased the sensitivity to noradrenaline, though carbachol, tyramine and amphetamine also caused small increases. These results are summarized in Table 6. Shanes (1958) has classified procaine as a " stabilizer " of cell membranes. Since raised potassium is known to depolarize membranes, its antagonism of procaine could equally well be explained on this basis.

Thus it may be concluded that there is little correlation between the ability of the substances tested to antagonize the nerve blocking action of guanethidine, low calcium, procaine or reserpine and their potentiating effect on noradrenaline. A possible exception to this generalization is the effect of carbachol against low calcium. Frank (1963) has shown that acetylcholine can mobilize bound calcium in skeletal muscle deprived of this ion. Douglas & Rubin (1963) have shown that it can permit the entry of calcium ions into the

adrenal medulla. Thus it is possible that carbachol may act similarly on the vas deferens in low calcium.

Several other points require comment. The experiments with amphetamine support the suggestion of Day & Rand (1963) that this drug competitively antagonizes guanethidine. It was shown that all the other antagonists of guanethidine produced a reversible effect, and, after washing out the bath, the sympathetic nerve block reappeared, even without adding more guanethidine (Fig. 1). However, after amphetamine had reversed the block, it did not return after washing out the bath (Fig. 3), suggesting that amphetamine, but not the other antagonist, had displaced guanethidine from its combination with the nerve.

The interactions of procaine and noradrenaline also need amplification. Boyd, Chang & Rand (1961) have reported that noradrenaline would not reverse a procaine block of the hypogastric nerve-vas deferens preparation. While this has been confirmed in the present paper, it has been shown that it does not hold for the transmurally stimulated preparation. In this case noradrenaline can both protect against and reverse a block by procaine. It seems likely therefore that procaine can block the ganglion in the hypogastric nerve (see Harvey, 1939) as well as depress transmission in the postganglionic sympathetic fibres. Noradrenaline, therefore, antagonizes the postganglionic effects of procaine, but not the preganglionic.

If the ability of the various substances to protect the electrically stimulated vas deferens against block by guanethidine, procaine, low calcium and previous treatment with reserpine does not depend on some increase in the sensitivity of the smooth muscle to the reduced amounts of transmitter still liberated, some other explanations must be sought. One other possibility also involving actions on the smooth muscle might be that the guanethidine antagonists increase muscle-to-muscle conduction in the vas deferens. If this were so, however, one would expect a closer correlation between the potency of the various substances as antagonists of guanethidine, procaine and low calcium with their ability to potentiate electrically induced contractions in both normal and reserpinized tissues.

There remains the possibility that the antagonizing action depends on an effect of these substances on the fine intramural nerve terminals, perhaps to facilitate the firing of action potentials. A similar effect of acetylcholine on sympathetic preganglionic fibres has been suggested (Volle & Koelle, 1961) and acetylcholine is known to depolarize vagal axons (Armett & Ritchie, 1960). However, it seems unlikely that such diverse substances as noradrenaline, arecoline, histamine and angiotensin would also have this effect.

SUMMARY

1. The ability of certain substances to reverse or prevent the action of guanethidine in blocking the effects of sympathetic nerve stimulation has been investigated in relation to the possibility that after blockade by guanethidine there might be release of sub-threshold quantities of transmitter from the sympathetic nerve, and that the antagonists of guanethidine might potentiate this reduced amount of transmitter.

2. Using the guinea-pig isolated vas deferens stimulated from an electrode in the lumen, a comparison was made of the ability of the various antagonists of guanethidine to potentiate added noradrenaline in the presence of guanethidine with their potency in reversing the sympatholytic action of guanethidine. Preparations were taken from reserpinized

guinea-pigs, or were treated *in vitro* with procaine or lowered calcium. These manœuvres are believed to reduce the output of transmitter from sympathetic nerves.

3. There was a poor correlation between the ability to potentiate added noradrenaline and to reverse the sympathetic blockade. Guanethidine itself potentiated the effects of added noradrenaline, and in its presence the other substances added but little to this increased sensitivity.

4. The effects of low calcium on nerve-induced responses were almost fully reversed by carbachol, and to a slightly lesser extent by amphetamine. However, neither of these substances potentiated added noradrenaline in low calcium solution.

5. The responses to electrical stimulation of reserpinized preparations were markedly increased by carbachol and histamine, but only slightly by raised potassium and not at all by amphetamine or tyramine. All these treatments potentiated the effects of added noradrenaline.

6. The only substances found to reverse the effects of procaine were raised potassium and, to a lesser extent, noradrenaline. Raised potassium was the most effective means found of potentiating added noradrenaline in the presence of procaine.

7. It is concluded that potentiation of residual transmitter cannot explain the reversal of the sympatholytic action of guanethidine, reserpine or low calcium by substances such as carbachol. It is possible that it could explain the reversal by high potassium of block due to procaine, though a depolarizing action on the nerve membrane seems an equally likely explanation.

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