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THE EFFECT OF PHENOXYBENZAMINE AND OF TOLAZOLINE ON THE RESPONSE TO SYMPATHETIC STIMULATION

BY

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The effect of phenoxybenzamine has been determined on the physiological response to sympathetic stimulation in two preparations, the rabbit isolated ileum and the guinea-pig isolated vas deferens. In both preparations phenoxybenzamine increased the response to stimulation of low frequency, the response being inhibitory in the one and motor in the other. This increase was large. As the stimulus frequency was raised, phenoxybenzamine caused a progressively smaller increase in the response, and at high frequencies phenoxybenzamine decreased the response. These observations agree with those of earlier workers who showed that antiadrenaline substances have more than one property. They not only block the motor effects of adrenaline and noradrenaline, but at the same time they may increase the response to sympathetic stimulation. The observations which have been made are not consistent with the interpretation which has been placed by others on the effect of phenoxybenzamine on the amount of noradrenaline appearing in the splenic vein following sympathetic stimulation; this interpretation assumes that phenoxybenzamine will decrease the response to sympathetic stimulation at low frequency. The mode of action of phenoxybenzamine is discussed, and fresh evidence that it has an anticholinesterase action is given.

In a series of papers (Brown & Gillespie, 1957; Brown, Davies & Gillespie, 1958; Brown, Davies & Ferry, 1961; Blakeley, Brown & Ferry, 1963) experiments have been described in which the postganglionic sympathetic fibres have been stimulated to an organ (mostly the spleen) in the cat and the amount of noradrenaline has been measured in the blood in the vein leaving the organ. The effect of antiadrenaline substances, in particular phenoxybenzamine and Hydergine (which may be described as dihydroergotoxine methanesulphonate), was determined, and it was observed that in their presence the output of noradrenaline was greatly increased when stimulation was applied at 10 shocks/sec, though at a higher frequency the increase in the output was much less. These results were interpreted by the authors to mean that the antiadrenaline substance did not alter the amount of noradrenaline released but prevented its destruction or uptake. They considered that the noradrenaline released by stimulation at a low frequency was either destroyed by the receptors or taken up by the tissue; it therefore did not appear in the vein. In the presence of the antiadrenaline substance, all the noradrenaline appeared in the vein.

The foregoing interpretation implied that, in the presence of the antiadrenaline substance, the stimulation was followed by a smaller physiological response, or by

no response at all. To test the conclusion of Brown and his colleagues, we have examined the effect of phenoxybenzamine on the response to stimulation in two preparations. We have used the rabbit isolated ileum in which stimulation of the periarterial nerves produces inhibition, and we have used the guinea-pig isolated vas deferens in which stimulation of the hypogastric nerve produces contraction. In both preparations we have compared the response to stimulation at various frequencies before and after phenoxybenzamine was added to the bath. In some experiments on the ileum we have used tolazoline also.

We have observed in both preparations that phenoxybenzamine increased the response to stimulation at low frequencies and decreased the response to stimulation at high frequencies.

METHODS

Ileum preparation with periarterial stimulation. Pieces of ileum with the attached mesentery were removed from a freshly killed rabbit. They were transferred to a beaker containing Locke solution or Tyrode solution, and the lumen was washed clean. They were then placed in fresh solution bubbled with 5% carbon dioxide in oxygen. One of the pieces was set up in an isolated organ-bath of 50 ml. capacity at 33° C. A silk thread was first tied to the main artery supplying the loop, and was passed through Saxby electrodes (Burn & Rand, 1960). The piece of artery was gently pulled inside the electrodes where it was firmly held. The ileum was attached by its upper end to a frontal writing lever. Maximal stimuli from a rectangular wave stimulator were applied at various frequencies, using a constant number of stimuli. The stimuli were of 1 msec duration.

Hypogastric nerve-vas deferens preparation. Guinea-pigs, 450 to 500 g of body weight, were killed by a blow on the head, the abdomen was opened in the midline and the distal colon was retracted to one side. The hypogastric nerves were dissected free. The vas deferens of each side was cut from its attachments to the epididymis at one end and the urethra at the other, and removed with the accompanying nerve. The preparation was mounted in a 150 ml. organ-bath containing McEwen's solution (1956) at 29° C, through which a stream of 5% carbon dioxide in oxygen was passed. The nerve was held in a pair of Saxby electrodes (Burn & Rand, 1960). Stimulation was applied at 2 min intervals. The shocks were rectangular, supramaximal, and of 0.5 msec duration. Contractions were recorded with a frontal lever magnifying about five-times.

The salts used were hyoscine hydrobromide, noradrenaline bitartrate, phenoxybenzamine hydrochloride (kindly supplied by Smith, Kline & French) and tolazoline hydrochloride, and the doses are given in terms of these salts, except that the dose of noradrenaline is given in terms of the base.

RESULTS

Observations on the rabbit ileum preparation in Locke solution. Fig. 1 shows the results of an experiment in which the periarterial nerves were stimulated with 500 shocks at various frequencies. Hyoscine (10^{-7} g/ml.) was present in the bath. Burn, Dromey & Large (1963) have shown that hyoscine increases the inhibition in response to stimulation at frequencies less than 10 shocks/sec, and, in this experiment, stimulation at 3 shocks/sec before hyoscine had been added failed to cause inhibition. As a rule hyoscine has no effect on the response to stimulation at 10 shocks/sec or greater frequencies. In the upper panels of Fig. 1 are the control responses, and in the lower panels those after the addition of phenoxybenzamine (3×10^{-6} g/ml.) to the bath. The response to the lowest frequency was increased

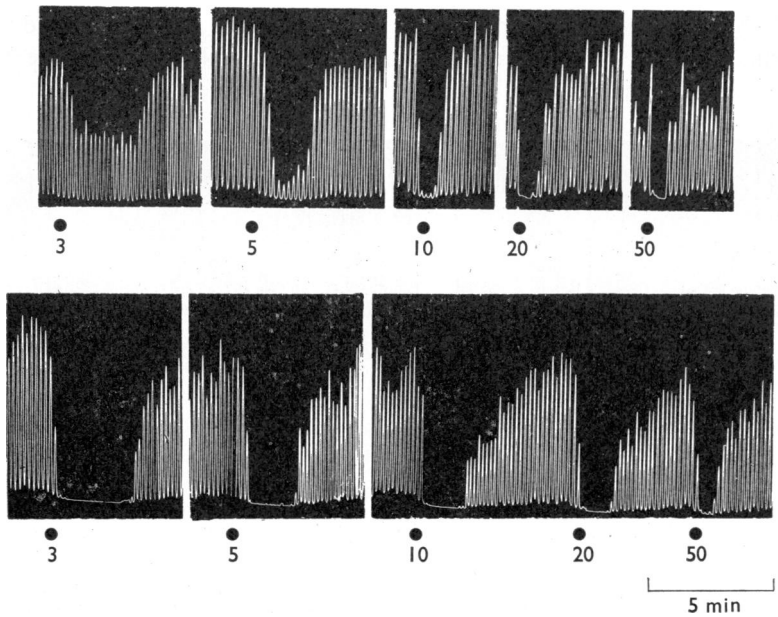

Fig. 1. Rabbit isolated ileum preparation in Locke solution. Stimulation of periarterial nerves in the presence of hyoscine (0.1 μg/ml.). Each stimulation was 500 shocks given at the frequency (shocks/sec) indicated below the record. Panels in the upper row show control responses. Panels in the lower row show responses in the presence of phenoxybenzamine (3×10^{-6} g/ml.). Note that phenoxybenzamine increased the response most at the frequency of 3 shocks/sec and progressively less as the frequency rose.

the most, and the responses to higher frequencies were increased progressively less, the increase of the response to 50 shocks/sec being small.

In this experiment the inhibition in response to each stimulation was measured by tracing the area of inhibition on paper, and then cutting out and weighing the paper. This was done twice, and the mean figures are shown in Table 1. The ratio of the areas in the presence of phenoxybenzamine to the control areas shows that the greatest increase in response to stimulation was at the lowest frequency and that the increase declined with rising frequency until there was almost no increase at 50 shocks/sec.

TABLE 1

NUMERICAL EXPRESSION OF AREAS OF INHIBITION IN RESPONSE TO PERIARTERIAL STIMULATION OF THE RABBIT ISOLATED ILEUM PREPARATION AT DIFFERENT FREQUENCIES BEFORE AND AFTER THE ADDITION OF PHENOXYBENZAMINE

Stimulus frequency (shocks/sec)	Area of inhibition: Control (C)	Area of inhibition: After phenoxybenzamine (P)	Ratio P/C
3	552	1,477	2·7
5	436	838	1·9
10	403	855	2·1
20	409	585	1·4
50	321	393	1·2

Fig. 2 shows a similar experiment in which hyoscine was not added; in this experiment, the addition of phenoxybenzamine (10 μg/ml.) increased the response to frequencies of 5 and 10 shocks/sec, but decreased the response to 50 shocks/sec.

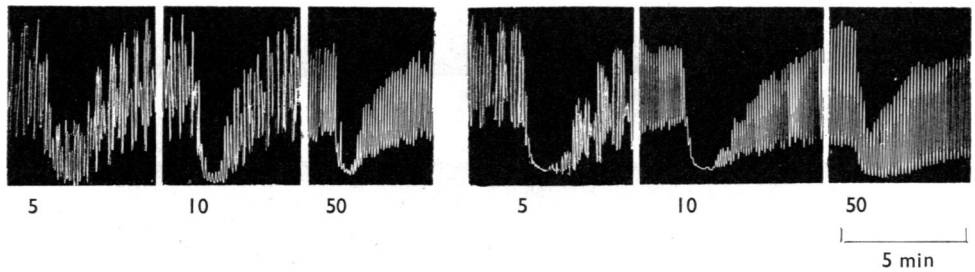

Fig. 2. Rabbit isolated ileum preparation in Locke solution. Stimulation as in Fig. 1, but hyoscine was not present. The left-hand panels show control responses to 500 shocks at the frequencies indicated below the records. The panels in the right-hand panel show responses in the presence of phenoxybenzamine (10^{-5} g/ml.). Note that phenoxybenzamine increased the response to stimulation at 5 and 10 shocks/sec but decreased the response to stimulation at 50 shocks/sec.

Fig. 3 is taken from another experiment without hyoscine. It shows that phenoxybenzamine increased the response to stimulation at 20 shocks/sec, but decreased the response to stimulation at 60 and 100 shocks/sec.

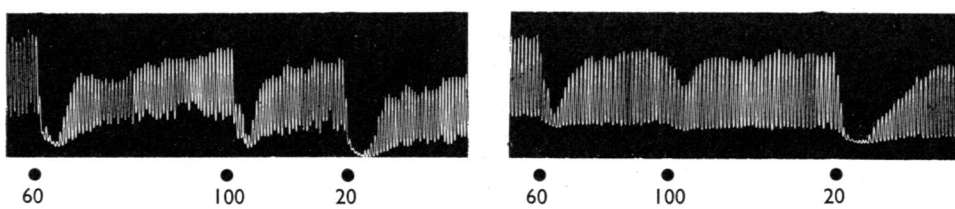

Fig. 3. Rabbit isolated ileum preparation in Locke solution. The left-hand panel shows control responses to periarterial stimulation at 60, 100 and 20 shocks/sec. The right-hand panel shows responses at the same frequencies 20 min after the addition of phenoxybenzamine (3×10^{-6} g/ml.). Note that phenoxybenzamine increased the response to 1,000 shocks at 20 shocks/sec but decreased the response to stimulation at 60 and 100 shocks/sec.

Experiments on the rabbit ileum preparation in Tyrode solution. The increase in the response to stimulation could be explained by the release of more noradrenaline, or by the release of the same amount of noradrenaline which in the presence of phenoxybenzamine exerted a greater effect. In a series of experiments we compared the response to stimulation with that to noradrenaline. One experiment is illustrated in Fig. 4. Hyoscine (10^{-7} g/ml.) was present. The panels on the left show the control responses and those on the right the responses in the presence of phenoxybenzamine (6×10^{-6} g/ml.); these were prolonged at 5 and 40 shocks/sec, and less so at 80 shocks/sec. The inhibition caused by noradrenaline was not increased when 0.5 μg was added to the 50 ml. organ-bath for 1 min. The same result was obtained in other experiments, but there were also experiments in which the inhibition produced by noradrenaline was greater after phenoxybenzamine.

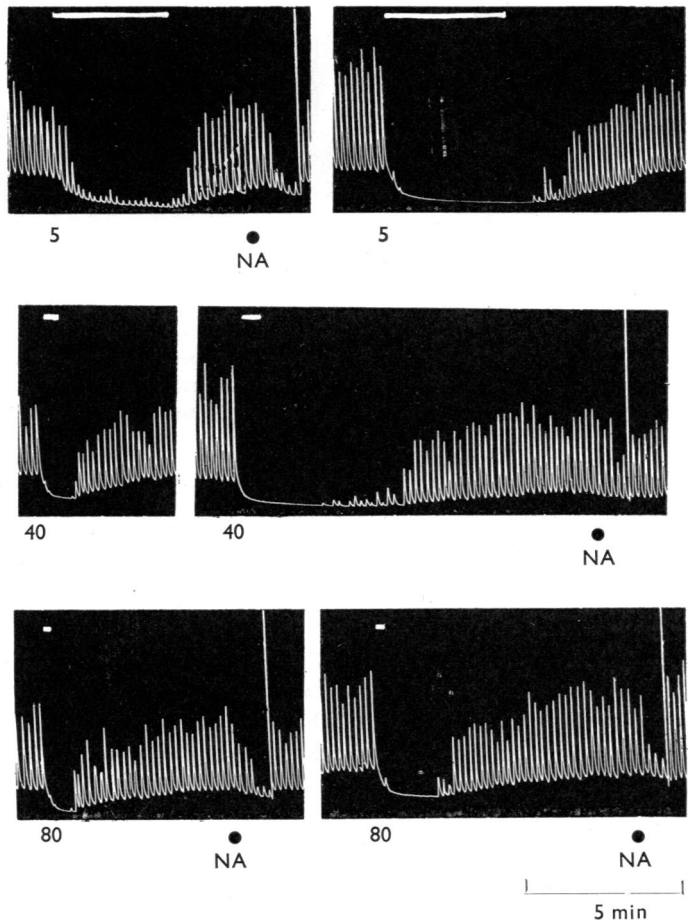

Fig. 4. Rabbit isolated ileum preparation in Tyrode solution. Hyoscine (0.1 μg/ml.) was present. The control responses are on the left, and the responses in the presence of phenoxybenzamine (6×10^{-6} g/ml.) are on the right. The effect of sympathetic stimulation (1,000 shocks) at the frequencies indicated and also the effect of noradrenaline (10^{-8} g/ml. at NA) are shown. Phenoxybenzamine increased the response at all three frequencies, though least at 80 shocks/sec. The effect of noradrenaline present in the bath for 60 sec was slightly diminished by phenoxybenzamine.

This increase was small compared with the increase in response to stimulation. There was no correspondence between the effect of phenoxybenzamine on the response to stimulation and on the response to noradrenaline. The former response was always increased at low frequencies and often decreased at high frequencies. The latter response was sometimes reduced or unaffected and sometimes increased.

Effect of tolazoline. Some experiments were made with tolazoline in Locke solution. One is illustrated in Fig. 5, in which the effect of stimulating the peri-arterial nerves at 5, 10 and 20 shocks/sec was increased by tolazoline (10^{-5} g/ml.), the effect of stimulating at 30 shocks/sec was not changed, and the effect of

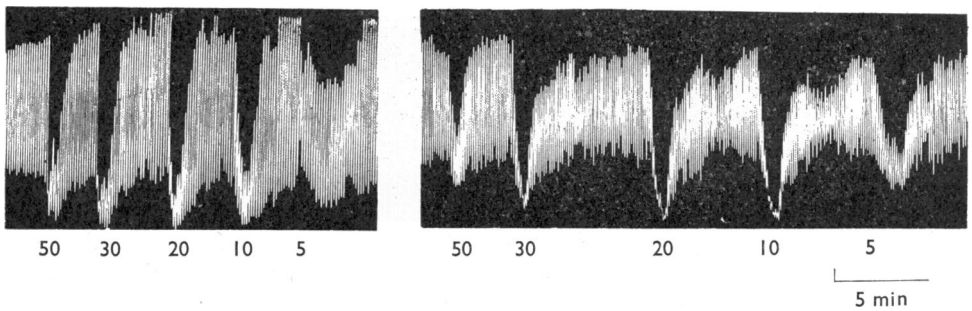

Fig. 5. Action of tolazoline (10^{-5} g/ml.) on the rabbit isolated ileum preparation. The left-hand panel shows control responses to 1,000 shocks applied to the periarterial nerves at the frequencies indicated below the record. Note that tolazoline increased the response to stimulation at 5, 10 and 20 shocks/sec, but had little effect at 30 shocks/sec. It decreased the response to 50 shocks/sec.

stimulating at 50 shocks/sec was reduced. The increased response at low frequencies and decreased response at high frequencies was observed in two other experiments.

Observations on the hypogastric nerve-vas deferens preparation. Burn & Weetman (1963) carried out experiments on the preparation devised by Huković (1961) of the guinea-pig vas deferens with the hypogastric nerves. Making observations in the presence of hyoscine (10^{-7} g/ml.) they observed that both physostigmine and neostigmine increased the response to stimulation at 5 or 10 shocks/sec, but decreased the response to stimulation at 20 shocks/sec. Mr Weetman has kindly made some observations in the same preparation with phenoxybenzamine, which are set out in Table 2. One experiment is shown in Fig. 6. Another experiment,

TABLE 2

PERCENTAGE CHANGE IN THE HEIGHT OF CONTRACTION OF THE VAS DEFERENS PREPARATION DUE TO PHENOXYBENZAMINE WHEN STIMULATION WAS APPLIED TO THE HYPOGASTRIC NERVE AT VARIOUS FREQUENCIES

Observations were made within 30 min (mostly 15 to 20 min) after adding phenoxybenzamine. Each concentration of phenoxybenzamine was used in one experiment

Stimulus frequency (shocks/sec)	Concentration of phenoxybenzamine (μg/ml.)				
	4	5	20	20	20
5	+800		+160	+1,200	
10	+36	+33	+18	+53	+127
20	+1	−75	−35	−40	−11
40					−23

for which we are indebted to Mr B. J. Large, is shown in Fig. 7. In all experiments the results were the same. Phenoxybenzamine acted like physostigmine and neostigmine; it increased the contraction in response to stimulation at a low frequency, and decreased it in response to stimulation at a higher frequency.

Phenoxybenzamine and tolazoline as anticholinesterases. In order to obtain further light on the view of Boyd, Chang & Rand (1960) that tolazoline and phenoxybenzamine possess anticholinesterase activity, we asked Dr F. Hobbiger to examine these compounds. He kindly did so, and has given us permission to quote his findings. He wrote that:

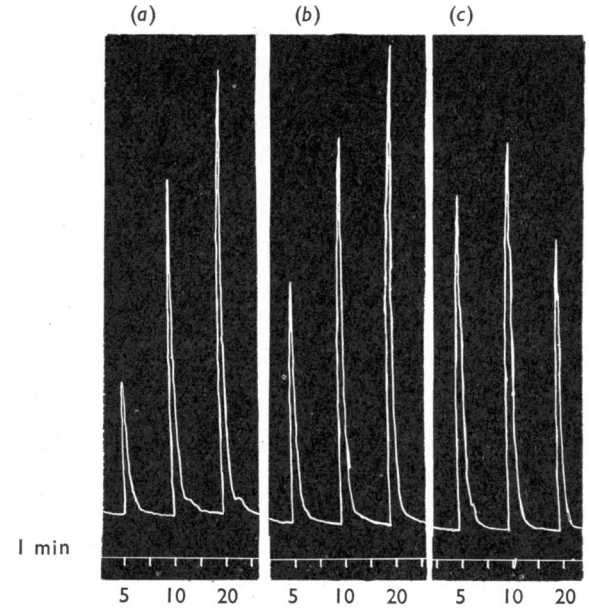

Fig. 6. Isolated hypogastric nerve-vas deferens preparation. Contractions in the presence of hyoscine (10^{-7} g/ml.), in response to 200 shocks at frequencies of 5, 10 and 20 shocks/sec applied to the hypogastric nerve. (*a*), control responses; (*b*), responses immediately after adding phenoxybenzamine (2×10^{-5} g/ml.) to the bath; (*c*), responses 10 min later. Note that phenoxybenzamine increased the response to all frequencies in (*b*), but decreased the response to 20 shocks/sec in (*c*). (Experiment of D. F. Weetman.)

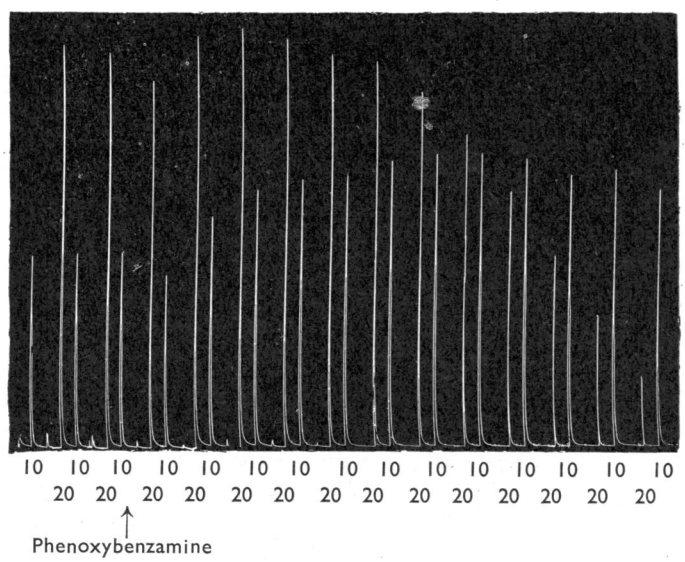

Fig. 7. Isolated hypogastric nerve-vas deferens preparation. Contractions in the presence of hyoscine (10^{-7} g/ml.) in response to 200 shocks at frequencies of 10 and 20 shocks/sec applied to the hypogastric nerve. Phenoxybenzamine (5×10^{-6} g/ml.) was added to the bath at the arrow. The main effect was an increase in the response to stimulation at 10 shocks/sec, and a decrease in the response to stimulation at 20 shocks/sec. (Experiment of B. J. Large.)

"The anticholinesterase activity was determined manometrically at 37° C in a medium of 0.025 M-sodium bicarbonate solution. As enzyme sources of acetyl- and butyrylcholinesterase, guinea-pig brain (in the presence of 0.1 mM-*iso*-OMPA to inhibit butyrylcholinesterase; substrate: 0.003 M-acetylcholine) and guinea-pig ileum (substrate: 0.003 M-butyrylcholine) respectively were used.

Under these conditions the dissociation constants (k_1) are:

	Acetylcholinesterase	Butyrylcholinesterase
Tolazoline	4.6×10^{-6} M	1.5×10^{-4} M
Phenoxybenzamine	1.9×10^{-5} M*	7×10^{-6} M

*This figure is an approximation since the limitation in solubility restricts the range of concentrations which can be used.

The difference between the antiacetylcholinesterase activities of the two substances is thus of the same order as that between their effectiveness in potentiating the response of the vas deferens to hypogastric stimulation (as observed by Boyd *et al.*, 1960). This is not the case with the antibutyrylcholinesterase activity.

Further experiments with physostigmine, etc., are necessary to establish whether or not the relationship between antiacetylcholinesterase activity and effectiveness in potentiating the response to nerve stimulation is purely coincidental." [*Iso*-OMPA is bis(*NN'*-isopropylphosphodiamidic) anhydride.]

DISCUSSION

It has long been commonly assumed that antiadrenaline compounds, including ergotoxine, tolazoline, phenoxybenzamine and Hydergine, possess one property, namely that of reducing or reversing the motor effects of adrenaline and noradrenaline. The natural expectation has been that these substances will also reduce or abolish the motor effects of sympathetic stimulation. There are, however, several observations that the response to sympathetic stimulation is increased, while that to adrenaline is decreased, by antiadrenaline substances. Bacq & Fredericq (1935) described a small increase in the contraction of the nictitating membrane caused by sympathetic stimulation after the injection of piperoxan, when the effect of adrenaline was decreased. Jang (1941), working with the perfused rabbit ear as prepared by Gaddum & Kwiatkowski (1938), showed that, when a concentration of piperoxan (5×10^{-8} g/ml.) was perfused through the ear, the effect of stimulating the postganglionic nerves in causing constriction was increased, while the effect of injecting adrenaline was diminished. He also injected yohimbine (1 mg/kg) into the spinal cat; this reversed the pressor effect of adrenaline, and completely suppressed its effect on the nictitating membrane, "but the effect of sympathetic stimulation on the membrane was only slightly diminished at first, and became much larger after a while." Jang obtained similar results with ergotoxine. After it was given, the effect of adrenaline on the nictitating membrane was almost absent, but the effect of sympathetic stimulation was greater than before. More recently, Varagić (1956a) made an isolated preparation of the rabbit uterus and stimulated the hypogastric nerves. He found that the motor response to sympathetic stimulation was increased by cocaine, which showed that the contraction was due to nor-

adrenaline, and he showed also that the response was increased by tolazoline. Huković (1959) prepared rabbit isolated atria with sympathetic nerves attached. He found that the effect of sympathetic stimulation on the atrial rate of beating was greatly increased by phenoxybenzamine. Boyd *et al.* (1960) examined the effect of several antiadrenaline agents on the response of the vas deferens of the guinea-pig to hypogastric nerve stimulation. They observed that tolazoline, phenoxybenzamine, yohimbine, ergotamine and piperoxan all increased the effect of stimulation when added to the bath in concentrations from 0.1 to 20 μg/ml. Gillespie & Mackenna (1961) found that " in the ileum both tolazoline and ergotamine tartrate enhanced the inhibitory effect of periarterial nerve stimulation if frequencies of 50/sec and above were used." Recently Kirpekar & Cervoni (1963) found that the relaxation of the isolated colon in response to stimulation of the lumbar colonic nerves at 2 shocks/sec was greatly increased by the addition of phenoxybenzamine (10^{-5} g/ml.) to the bath.

These observations by other workers and those described in this paper show that the idea that antiadrenaline substances have only one property is incorrect. Their action is not confined to reducing or abolishing the motor effects of adrenaline and noradrenaline. In addition they increase the response to sympathetic stimulation at low frequency both when the response is motor and when it is inhibitory.

The question which then arises is how far the increase which we observed was due to an increased liberation of noradrenaline, and how far it was due to an increase in the effect of the same amount of noradrenaline. For example, Holzbauer & Vogt (1955) described a large increase in the action of adrenaline on the rat uterus when phenoxybenzamine was present. The work of Stafford (1963) has shown that there is much difference between different organs. Phenoxybenzamine and guanethidine have an action like cocaine, and potentiate the effect of noradrenaline on rabbit atria eight- and seven-times respectively. However in the rabbit duodenum she observed that phenoxybenzamine decreased the effect of noradrenaline. We obtained results which varied. In some experiments (see Fig. 4) in which the response to stimulation was much increased, we observed no change; in others there was an increase in the response to noradrenaline, but this always appeared to be insufficient to account for the increased response to stimulation. More significant was our observation that the increase in the response to stimulation depended on the frequency, being greatest at the lowest frequency, and that, as the frequency rose, the increase became steadily less. Often the response was decreased at frequencies above 50 shocks/sec. If phenoxybenzamine acted by increasing the effect of noradrenaline, the response should have been increased at all frequencies.

Our observations appeared to be, in general, parallel to the observations of Brown and his colleagues on the output of noradrenaline in the splenic vein. Phenoxybenzamine affected both in the same way, increasing the inhibition of the ileum and the output of noradrenaline in the splenic vein most at the lowest frequency, becoming less and less effective as the frequency rose, and decreasing them both at the high frequencies. This suggests that phenoxybenzamine produced the changes by altering the amount of noradrenaline released from the nerve endings.

It greatly increased the amount liberated at low frequencies, the increase being progressively less as the frequency rose, until at the high frequencies it diminished the amount liberated. The same changes were recorded when the hypogastric nerves to the vas deferens were stimulated. These effects were obtained with concentrations of phenoxybenzamine which may have been lower than those used in the experiments in which the output of noradrenaline was measured, but, if they were, there is no reason to suppose that higher concentrations of phenoxybenzamine would not have the same effect on the liberation of noradrenaline. We are thus able to give a different interpretation to the experiments on noradrenaline output, and this is our main purpose.

We must however consider how phenoxybenzamine produces its effect, even though the conclusions which are drawn on this second point are tentative. One explanation of the increase in the effect of sympathetic stimulation in the ileum is that phenoxybenzamine acts like cocaine. This, however, would not explain why phenoxybenzamine decreased the effect at high frequencies. Kirpekar & Cervoni (1963) observed that cocaine (10^{-5} g/ml.) increased the response of the isolated colon to stimulation of the lumbar colonic nerves at 2 shocks/sec, but this increase was much less than that caused by phenoxybenzamine in the same concentration. These workers also observed that cocaine increased the output of noradrenaline in the splenic vein in response to stimulation of the splenic nerves at 10 shocks/sec, the increase being seen in sixteen out of nineteen experiments. This increase was, however, much smaller than the increase they observed after the injection of phenoxybenzamine.

Another explanation of the action of phenoxybenzamine is that it may be an anticholinesterase. This was suggested by Boyd *et al.* (1960) who observed that the contractions of the isolated vas deferens in response to hypogastric stimulation were increased by antiadrenaline substances including phenoxybenzamine, and also by physostigmine.

In manometric experiments they measured the rate of destruction of acetylcholine by an extract of guinea-pig vas deferens, determining the effect of the antiadrenaline substances upon it. All were found to have an inhibitory action, tolazoline being the most active and phenoxybenzamine the least.

Rothlin (1923) showed that ergotamine increased and prolonged the effect of vagal stimulation in the rabbit, and also the depressor action of acetylcholine in the dog. Loewi & Navratil (1926) used ergotamine with the intention of excluding the sympathetic action when stimulating the vagosympathetic trunk to the frog heart. They found that ergotamine had an unexpected effect in greatly prolonging the effect of vagal stimulation and of "Vagusstoff." Ergotamine, like physostigmine, inhibited the splitting of acetylcholine *in vitro* by the esterase present in heart extract. Ergotamine, like physostigmine, failed to modify the action of choline, pilocarpine and muscarine. They said: "Es wirkt also Ergotamin ganz wie Physostigmin."

Matthes (1930) showed that both ergotoxine and ergotamine inhibited the cholinesterase in the blood, but were much weaker than physostigmine. Gowdey (1948)

showed that tolazoline potentiated the action of acetylcholine on the guinea-pig ileum, and we have observed a potentiation on the frog rectus.

Recently Osswald & Guimaraes (1962) observed that ergotoxine increased the depressor effect of acetylcholine in the dog, and attributed this effect to the anti-cholinesterase action of the ergot alkaloids. Brown *et al.* (1961) found that bilateral vagotomy was necessary before giving Hydergine "to abolish the vagal inhibition of the heart otherwise produced by the drug." Hydergine is the methanesulphonate of the dihydro derivative of what used to be called ergotoxine.

The observations made by Dr Hobbiger confirm the evidence of Boyd *et al.* (1960) that tolazoline and phenoxybenzamine have an anticholinesterase action, though, as Dr Hobbiger says, further experiments are required before it can be said that the potentiation of the response to sympathetic stimulation is due to this action. The observations illustrated in Figs. 6 and 7 are, however, in favour of this concept. They show that phenoxybenzamine had the same effect as did physostigmine and neostigmine on the vas deferens. All three increased the effect of stimulation at low frequency and decreased the effect of stimulation at high frequency. A similar action of physostigmine and neostigmine is well known at the neuromuscular junction in skeletal muscle, and it can scarcely be doubted that phenoxybenzamine produces such an effect in the hypogastric nerve-vas deferens preparation by acting as an anticholinesterase at some point. What must be considered is the possibility that the antiadrenaline substances exert an anticholinesterase action more powerfully in the terminations of sympathetic postganglionic fibres than elsewhere, and in some cases more powerfully than physostigmine and neostigmine. Thus Varagić (1956a) observed that, whereas tolazoline increased the effect of hypogastric nerve stimulation on the rabbit uterus, he obtained a similar effect with physostigmine in only two out of fourteen experiments. In the colon Varagić (1956b) found that, in the presence of tolazoline, sympathetic stimulation caused an immediate contraction preceding the usual inhibition; physostigmine rarely had this effect, but tolazoline was potentiated by physostigmine.

Other experiments show that physostigmine exerts an effect at sympathetic post-ganglionic endings. Thus Burn, Rand & Wien (1963) found that physostigmine and neostigmine, in the presence of hyoscine, increased the effect of stimulating the postganglionic fibres to the nictitating membrane, the increase being greatest for stimulation at the lowest frequency. Dirnhuber & Cullumbine (1955) and also Varagić (1955) showed that physostigmine increased blood pressure in the rat. Gokhale, Gulati & Joshi (1963) have found that this rise is completely blocked by bretylium and guanethidine, but only partially by hexamethonium even in very large doses. The effect of physostigmine is therefore exerted through the sympathetic nerves and perhaps in part at the sympathetic postganglionic terminations.

Blakeley *et al.* (1963) have concluded that the effects of phenoxybenzamine and Hydergine on the output of noradrenaline in the splenic vein following stimulation of the splenic nerves are not due to an anticholinesterase action of these substances. While agreeing that their evidence appears at first sight to justify this conclusion, we think that there are good reasons for suspending judgment on this.

The thanks of one of us (J. H. B.) are due to Dr O. H. Lowry for the opportunity of working in this Department. We wish also to thank Dr F. Hobbiger for permission to quote his results, Mr D. F. Weetman for Fig. 6 and Mr B. J. Large for Fig. 7. This work was supported in part by a grant (5T1-NB-5221) from the National Institute of Neurological Diseases and Blindness, U.S.A.

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