

## EVIDENCE FOR A COMPETITIVE ANTAGONISM OF GUANETHIDINE BY DEXAMPHETAMINE

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After guanethidine had blocked the response of the cat nictitating membrane to sympathetic nerve stimulation, dexamphetamine restored the responses to all frequencies of stimulation. Dexamphetamine antagonized the sympathetic nerve block by guanethidine in the isolated sympathetically innervated rabbit ileum; the evidence suggests that the antagonism was competitive. Dexamphetamine antagonized the sympathetic nerve block by guanethidine in the isolated hypogastric nerve-vas deferens preparation of the guinea-pig. Doses of dexamphetamine, larger than those required to antagonize the blocking action of guanethidine, abolished the responses of the nictitating membrane, ileum and vas deferens to nerve stimulation. Dexamphetamine did not influence the depletion of noradrenaline by guanethidine in the heart and spleen of rabbits. The hypothesis is advanced that both dexamphetamine and guanethidine act on the store of noradrenaline at sympathetic nerve endings.

Day (1962) showed that dexamphetamine and other indirectly acting sympathomimetic amines prevent the onset of the sympathetic nerve block by guanethidine, bretylium and xylocholine. If the nerve block is first established, then dexamphetamine will reverse it. This drug antagonism was demonstrated with the sympathetically innervated isolated rabbit ileum *in vitro* and the cat nictitating membrane *in vivo*. Day & Rand (1962) extended these observations to include the sympathetically mediated pressor responses produced reflexly by stimulation of the central end of a divided vagus nerve and by occlusion of the common carotid arteries in anaesthetized cats and dogs.

Day & Rand (1962) pointed out that, since sympathetic nerve blocking drugs and indirectly acting sympathomimetic amines have properties in common (see Discussion), they might act at the same site: the site suggested was the store of noradrenaline at sympathetic nerve endings. This paper deals with a further examination of the interaction between guanethidine and dexamphetamine to test the hypothesis that these drugs act at the same site.

### METHODS

*Cat nictitating membrane.* Cats were anaesthetized with a mixture of chloralose (80 mg/kg of body weight) and pentobarbitone (6 mg/kg) given intraperitoneally. Contractions of the right nictitating membrane were measured with an isotonic frontal writing lever (magnification 10:1) exerting a tension of 3 g on the membrane. For nerve stimulation the postganglionic fibres from the superior cervical ganglion were dissected free, laid across bipolar platinum

electrodes and covered with liquid paraffin. Electrical stimulation was with rectangular wave pulses of supramaximal strength and 2.0 msec duration, at frequencies ranging from 0.1 to 100 shocks/sec, and for periods of 12 sec applied at intervals of not less than 2 min.

*Isolated rabbit ileum with sympathetic nerves.* This preparation was prepared as described by Day & Rand (1961) using McEwen's (1956) solution at 37° C. The sympathetic nerves were stimulated with rectangular wave pulses of supramaximal strength, 2.0 msec duration, at 50 shocks/sec applied for 12 sec in every 2 min. The stimulation time was kept constant by an automatic timing device. In nearly all preparations stimulation with these parameters completely inhibited pendular movements; the few preparations that responded to sympathetic nerve stimulation with a less pronounced relaxation were discarded.

In nearly all experiments more than one preparation (usually 4-16) were made from each rabbit. Those preparations not used immediately were stored in McEwen's solution at room temperature and constantly gassed with a mixture of 5% carbon dioxide and 95% oxygen. Preparations stored in this way for up to 7 hr almost always retained their responsiveness to nerve stimulation and to drugs.

*Guinea-pig vas deferens with hypogastric nerve.* This preparation was made as described by Huković (1961), using McEwen's solution at 32° C in a 50 ml. bath. The hypogastric nerve was stimulated with rectangular wave pulses of supramaximal strength, 2 msec duration, at a frequency of 20 shocks/sec, and for 5 sec every 2 min.

*Estimation of noradrenaline content of tissues.* This was done with the spectrofluorometric methods of Cass & Spriggs (1961).

*Drugs.* The drugs used were guanethidine sulphate, dexamphetamine sulphate, noradrenaline bitartrate, bretylium tosylate and cocaine hydrochloride; the amounts stated refer to the salts.

## RESULTS

### *Cat nictitating membrane*

In previous experiments (Day, 1962; Day & Rand, 1962) the blocking action of guanethidine and its reversal by dexamphetamine were studied using only one frequency of nerve stimulation. In the present experiments, the responses to a range of stimulus frequencies were recorded. The results from an experiment are illustrated in Fig. 1. Guanethidine (5 mg/kg, intravenously) completely abolished the responses of the membrane to low stimulus frequencies (less than 2 shocks/sec) and greatly depressed responses to stimulation at higher frequencies (Fig. 1, —○—). These observations were made 45 min after injecting guanethidine, at which time the depressed responses to stimulation were constant. The sympathetic nerve block by guanethidine is very persistent; no recovery occurs in an acute experiment, and Page & Dustan (1959) and Maxwell, Plummer, Schneider, Povalski & Daniel (1960) found that the action of guanethidine persisted for 5 to 20 days after injection. Injection of dexamphetamine (0.5 mg/kg, intravenously) produced a conspicuous and persistent contraction of the membrane of normal cats; when given after guanethidine the drug produced only a slight contraction, but the responses to all stimulus frequencies gradually increased and were constant at their new levels after about 90 min. The restored responses (Fig. 1, —+—) were recorded 112 min after injecting dexamphetamine. In the experiment illustrated the contractions after guanethidine and then dexamphetamine were restored almost to the heights of the initial responses at all stimulus frequencies. The responses were measured from their own base-line, which was about 1 cm above the base-line (on the kymograph) at the start of the experiment, because of the slightly increased tone caused by dexamphetamine. A second

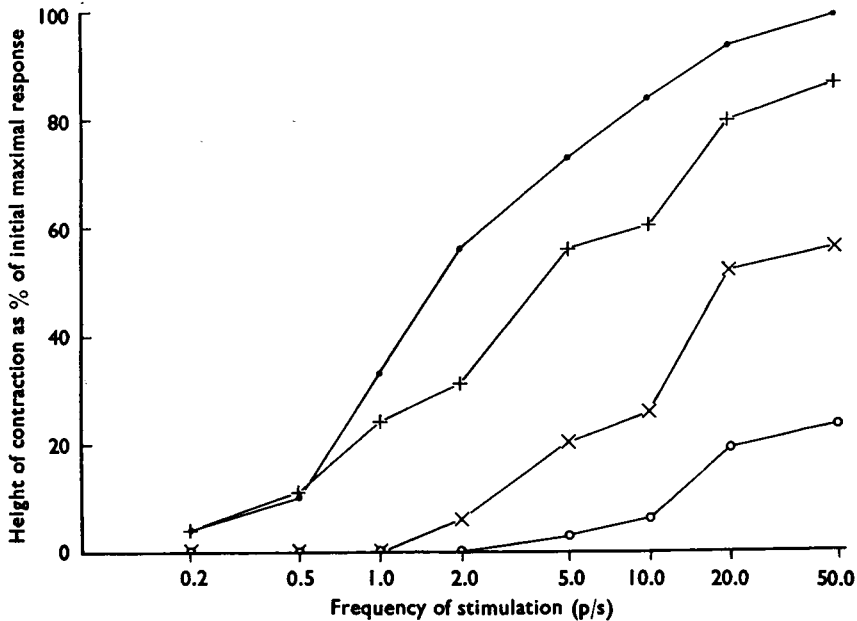


Fig. 1. Contractions of a cat nictitating membrane in response to stimulation of the postganglionic sympathetic nerve at various frequencies, shown as pulses/sec (p/s) on abscissa. The contractions are expressed as a percentage of the initial maximal contraction (100%  $\equiv$  70 mm on kymograph). —●—, initial observations; —○—, 45 min after guanethidine (5 mg/kg); —+—, 112 min after dexamphetamine (0.5 mg/kg), the contractions are almost restored; —x—, contractions after a further dose of guanethidine (5 mg/kg).

injection of guanethidine (5 mg/kg) was given, but now the drug had caused lesser block than the first injection. Similar results to those shown in Fig. 1 were obtained in five experiments with guanethidine (1, 2, 2, 2.5 and 5 mg/kg) and dexamphetamine (0.2, 0.5, 0.5, 0.5 and 0.5 mg/kg), and in two experiments with bretylium (10 and 10 mg/kg) and dexamphetamine (0.3 and 0.5 mg/kg). The optimum dose of dexamphetamine for reversing sympathetic nerve blockade due to guanethidine or bretylium was approximately 0.5 mg/kg. Higher doses of dexamphetamine were not more effective.

The effect of dexamphetamine alone on the responses of the membrane to nerve stimulation was studied in four experiments. The results from one are shown in Fig. 2. In these experiments the doses of dexamphetamine were, at the start of the experiments, very small (10 to 20  $\mu$ g/kg), and they were gradually increased on successive injections. In this way large and sustained contraction of the membrane was avoided, some tachyphylaxis being established by the smaller doses. Small doses of dexamphetamine (up to 20  $\mu$ g/kg) potentiated responses to stimulation at all frequencies. After larger doses the responses decreased. Thus, 0.48 mg/kg of dexamphetamine, the optimal dose for reversal of guanethidine blockade, greatly reduced contractions due to nerve stimulation (Fig. 2), except at a stimulus frequency of 100 shocks/sec when the response was the same as the control. Increasing the dose of dexamphetamine to about 5 mg/kg resulted in a stimulus frequency/

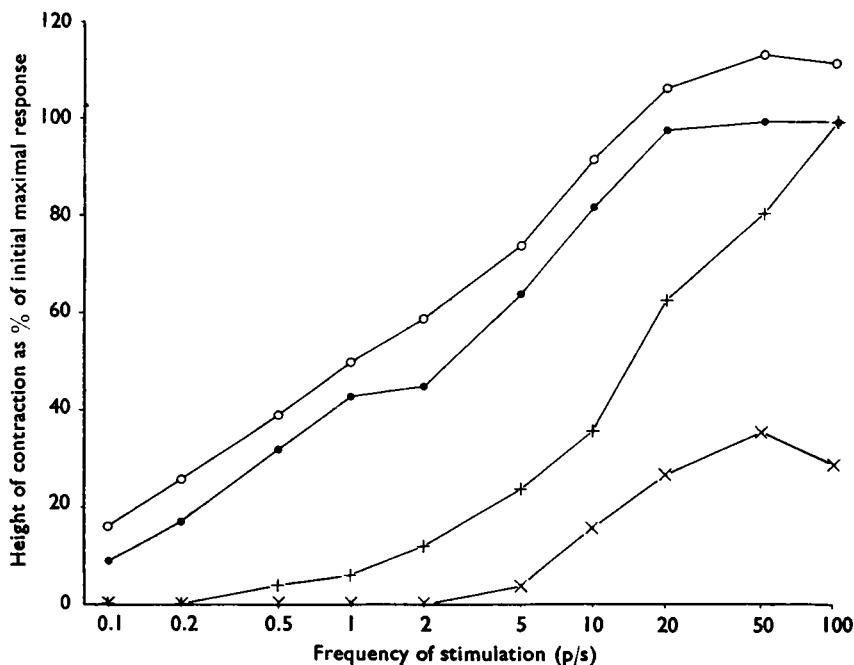


Fig. 2. Effect of dexamphetamine on the contractions of a cat nictitating membrane induced by postganglionic sympathetic nerve stimulation at various frequencies (p/s=pulses/sec, abscissa). The contractions are expressed as a percentage of the initial maximal contraction (100%  $\equiv$  78 mm on kymograph). —●—, initial observations; —○—, 20 min after dexamphetamine (10  $\mu$ g/kg), there is a slight potentiation of contractions; —+—, 30 min after dexamphetamine (0.48 mg/kg), there is blockade of responses; —x—, 30 min after dexamphetamine (5.48 mg/kg), there is marked blockade.

response relationship resembling that produced by guanethidine; however, in this case the tone of the membrane was increased. Nevertheless the responses of the membrane to stimulation were clearly impaired, because the peak height of the contraction was considerably less than the height of the maximal contraction at the start of the experiment. Dexamphetamine did not block the responses of the blood pressure and of the nictitating membrane to injected noradrenaline.

#### *Responses of rabbit ileum to sympathetic nerve stimulation*

This preparation offered a number of advantages for a more detailed quantitative analysis of the interaction between guanethidine and dexamphetamine. Several lengths of ileum could be taken from one rabbit, and the responses of different lengths to drugs were similar. Guanethidine blocked the inhibition of pendular movements caused by sympathetic nerve stimulation. With any dose of guanethidine which initially produced a detectable blockade, this blockade increased until it was complete. The rate of development of the blockade was proportional to the concentration of guanethidine in the bath. A suitable index for comparing the effectiveness of various doses of guanethidine was the time taken until sympathetic nerve

stimulation inhibited pendular movement by 50% ; however, observations were always continued beyond this time in order to be sure of the determination.

The relationship between the concentration of guanethidine in the bath and the time to achieve 50% blockade is shown in Fig. 3, A. Dexamphetamine (0.1  $\mu\text{g}/\text{ml}$ ., Fig. 3, B, and 0.5  $\mu\text{g}/\text{ml}$ ., Fig. 3, C) shifted the concentration/“ effectiveness ” curves

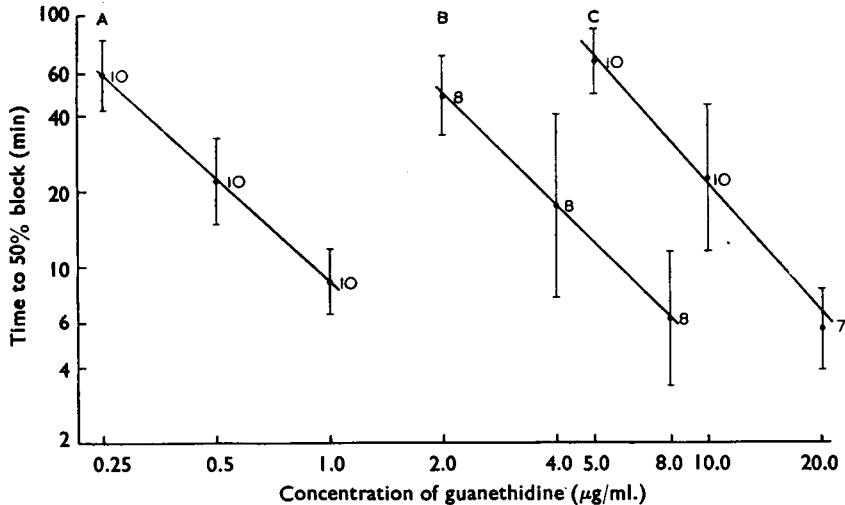


Fig. 3. Effect of guanethidine in blocking the response of the rabbit isolated ileum to sympathetic nerve stimulation. The ordinate shows the time taken to depress the response of the ileum to the point where nerve stimulation causes 50% inhibition of pendular movements; these times are plotted on a logarithmic scale. The concentrations of guanethidine in the bath are plotted on the abscissa on a logarithmic scale. A is the concentration/“ effectiveness ” curve for guanethidine alone, B is the curve for guanethidine in the presence of dexamphetamine (0.1  $\mu\text{g}/\text{ml}$ .) and C is the curve in the presence of dexamphetamine (0.5  $\mu\text{g}/\text{ml}$ .). Each point is the mean determined from the number of experiments indicated by the adjacent number; the standard deviations of each set of observations are indicated by the vertical lines. The sloping lines are the calculated regression lines.

for guanethidine to the right, indicating antagonism of the block induced by guanethidine. Fig. 3 has log/log co-ordinates; the calculated regression lines are very highly significant ( $P < 0.001$ , by analysis of variance), and the lines A, B and C do not differ significantly from parallel ( $P > 0.2$ , by analysis of variance). The regression lines were not significant when the time to 50% blockade was plotted against the log concentration of guanethidine.

In examining these data it is convenient to consider guanethidine as an agonist causing failure of response to sympathetic nerve stimulation. Reuse (1948) proposed the term “agonist” to describe an active drug; the word “antagonist” was then used to describe a drug which suppresses the action of an agonist. In the context of our experiments, guanethidine is the active drug (agonist) and dexamphetamine, since it suppresses the action of guanethidine, is the antagonist. Arunlakshana & Schild (1959) pointed out that, if an antagonist shifts the dose/response line of an agonist without altering the slope of the line (as in Fig. 3), this effect is consistent

with, but does not prove, competitive antagonism. They further showed how data of this type can be examined more precisely to see whether they fit the hypothesis of competitive antagonism. The plot of  $\log(x-1)$  against the negative  $\log B$  (where  $x$  is the ratio of the dose of guanethidine alone to the dose in the presence of a concentration,  $B$ , of dexamphetamine, when each dose produces the same effect) gives a line whose slope is equal to the value of  $pA_2 - pA_{10}$ . The intercept of this line with the abscissa gives the  $pA_2$  value for the antagonist. Under the conditions of competitive antagonism,  $pA_2 - pA_{10} = 0.95$ .

The results from seven sets of determinations are plotted in Fig. 4, in which the line is the calculated regression line. It is very highly significant ( $P < 0.001$ ). The regression coefficient is 1.02, which is in good agreement with the theoretical value of 0.95 for competitive antagonism. This agreement indicates that the antagonism of guanethidine by dexamphetamine may be competitive. The  $pA_2$  value for dexamphetamine in antagonizing guanethidine is 6.8. This value is somewhat smaller than those given by Arunlakshana & Schild (1959) for highly specific antagonisms such as that of atropine for acetylcholine, but it nevertheless suggests a reasonably high specificity. It has been pointed out (R. P. Stephenson, personal communication) that the analysis of Arunlakshana & Schild (1959) is based on the

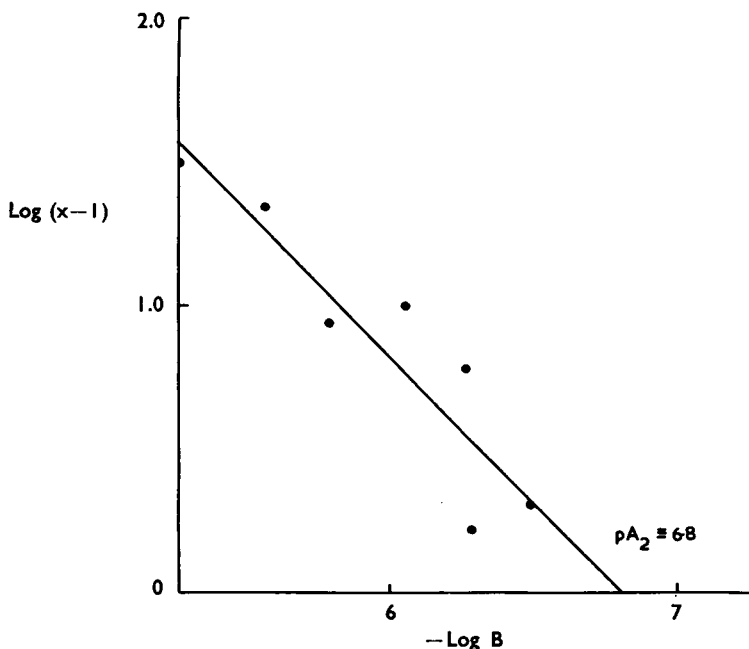


Fig. 4. Data such as those shown in Fig. 3 plotted by the method of Arunlakshana & Schild (1959) to determine the value of  $pA_2$  and  $pA_2 - pA_{10}$ . The ordinate is  $\log(x-1)$ , where  $x$  is the ratio of equiactive doses of guanethidine in the absence and in the presence of dexamphetamine. The abscissa is negative  $\log B$ , where  $B$  is the molar concentration of dexamphetamine. The line is the calculated regression line. It intersects the abscissa at 6.8, which is the  $pA_2$  value. The slope of the regression line is 1.02. For competitive antagonism of guanethidine by dexamphetamine the theoretical slope is 0.95 ( $= pA_2 - pA_{10}$ ).

presence of equilibrium conditions, that is, on the final effect produced by the drugs, whereas our analysis uses the time to produce an effect, which might be influenced by rate of diffusion or other factors. The time for guanethidine to block is a function of the concentration of guanethidine, and the theoretically preferable measurement of the size of the final effect of guanethidine is impractical with the sympathetically innervated ileum preparation, because there is eventually a complete block with even the lowest concentration of guanethidine which has any effect at all. It seems unlikely that small amounts of dexamphetamine would alter the rate of diffusion of guanethidine, but competition by dexamphetamine for receptors would decrease the rate of combination of guanethidine with receptors.

In similar sets of experiments the antagonisms of the blocking action of guanethidine by cocaine, and of bretylium by dexamphetamine, were examined. The relationship between bretylium and dexamphetamine was consistent with a competitive antagonism. The concentration/"effectiveness" curves for bretylium were shifted to the right, but remained parallel, in the presence of dexamphetamine. The plot of the results from five sets of experiments, treated in a similar way to that shown in Fig. 4, gave a value for  $pA_2 - pA_{10}$  of 0.88. The  $pA_2$  value was 7.0.

The relationship between guanethidine and cocaine was more complex. The concentration/"effectiveness" curve for guanethidine was steeper in the presence of cocaine. The plot of the data to yield the values for  $pA_2$  and  $pA_{10}$  was made on the basis of concentrations of guanethidine which caused 50% blockade in 26 min. The  $pA_2$  value was 7.8, but the value of  $pA_2 - pA_{10}$  was low, being 0.63. A low value for  $pA_2 - pA_{10}$  occurs when the antagonist has a paradoxical potentiating effect (Arunlakshana & Schild, 1959). The application of this analysis to our experiments is that high concentrations of cocaine block the response to nerve stimulation (Day, 1962). A further complication is the fact that low concentrations of cocaine sometimes increase the response to nerve stimulation. Both these factors are operating together to depress the value of  $pA_2 - pA_{10}$ .

Doses of dexamphetamine which, when given alone to the ileum, antagonized the blocking action of guanethidine neither decreased nor increased the response to nerve stimulation. Greater concentrations of dexamphetamine (30 to 100  $\mu\text{g}/\text{ml}$ .) blocked the response to nerve stimulation. In the presence of intermediate concentrations of dexamphetamine, which produced some impairment of the response to nerve stimulation, a further addition of the usual blocking dose of guanethidine did not cause a greater blockade.

#### *Isolated vas deferens with hypogastric nerve*

In this preparation also, the sympathetic nerve block by guanethidine was reversed by dexamphetamine. However, many substances, including smooth muscle stimulants, potentiate the contractions of the vas deferens in response to nerve stimulation (Huković, 1961; Sjöstrand, 1961), even after guanethidine blockade. The antagonism of guanethidine blockade by dexamphetamine can be readily distinguished from the relatively unspecific potentiation of responses to nerve stimulation by other substances, such as noradrenaline. Fig. 5 illustrates an experiment in which, when guanethidine (10  $\mu\text{g}/\text{ml}$ .) had blocked the responses, noradrenaline

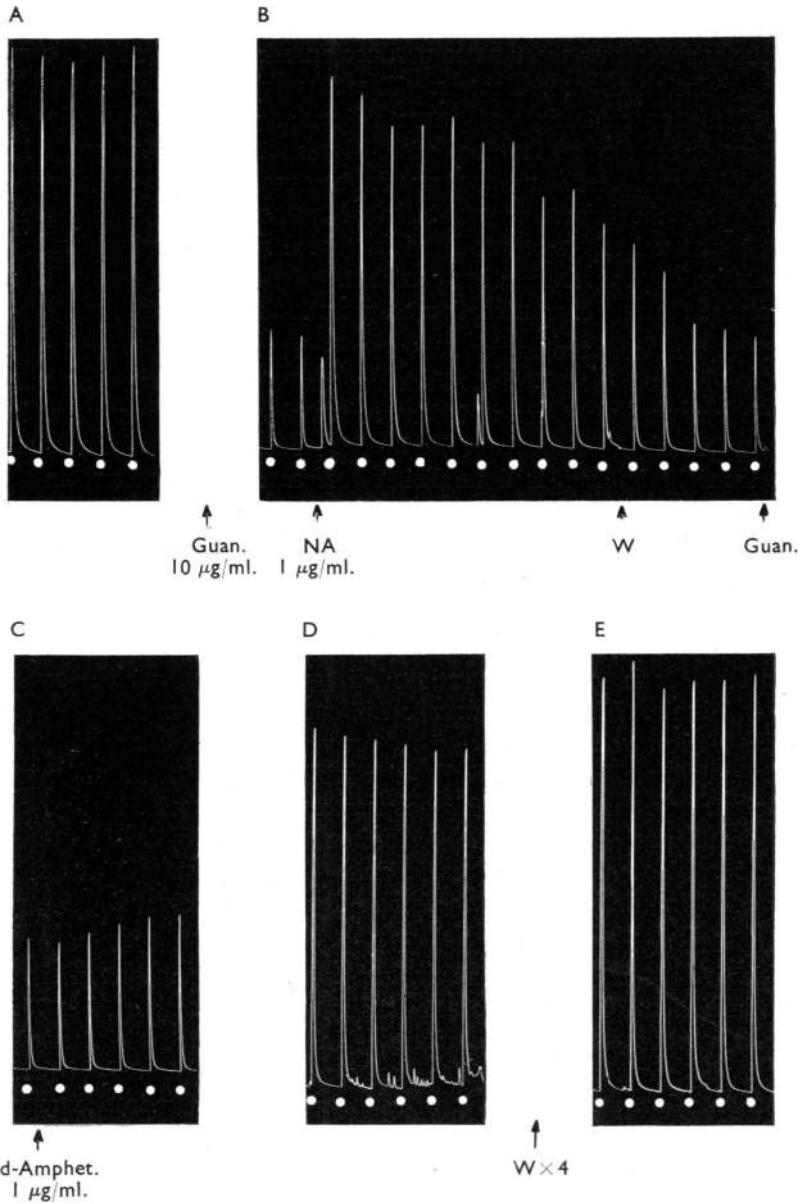


Fig. 5. Contractions of guinea-pig isolated vas deferens in response to hypogastric nerve stimulation. Initial observations are shown in A. Between A and B guanethidine ( $10 \mu\text{g/ml.}$ ) was added to the bath, and B shows the depressed contractions 75 min later. Noradrenaline (NA,  $1 \mu\text{g/ml.}$ ) was added to the bath and washed out at W. It caused an immediate but transient potentiation of responses. Guanethidine was replaced in the bath, and in C dexamphetamine ( $1 \mu\text{g/ml.}$ ) was added to the bath; it caused a slowly developing increase in the responses. D is 105 min after dexamphetamine. The gap between D and E was 20 min, during which time the bath was washed four times. The restoration of responses to nerve stimulation persisted.



(1  $\mu\text{g}/\text{ml}$ .) temporarily potentiated the small responses. After washing out the bath all traces of the potentiation disappeared. Guanethidine was replaced in the bath, and then dexamphetamine (1  $\mu\text{g}/\text{ml}$ .) was added. The responses gradually increased, and the increase persisted after repeatedly washing out the bath.

In experiments where dexamphetamine (1  $\mu\text{g}/\text{ml}$ .) alone was given to the vas deferens, there was a slight potentiation of the responses to nerve stimulation. Greater concentrations of dexamphetamine (40 to 100  $\mu\text{g}/\text{ml}$ .) greatly reduced the responses.

*Effect of dexamphetamine on the depletion of noradrenaline by guanethidine*

Guanethidine (12.5 mg/kg) depletes the catechol amine content of the hearts and spleens of rabbits (Cass, Kuntzman & Brodie, 1960). This has been confirmed. Dexamphetamine (0.5 mg/kg) was given to six rabbits, 18 hr after guanethidine. Pairs of these rabbits were killed 6, 24 and 48 hr later. The contents of noradrenaline in the heart and spleen were no different from those in rabbits which had had guanethidine alone for the same length of time. In another experiment, a rabbit was given dexamphetamine (2 mg/kg) 1 hr before guanethidine (12.5 mg/kg). The content of noradrenaline in the heart was the same as that in a litter mate given guanethidine without previous treatment with dexamphetamine.

#### DISCUSSION

The evidence presented suggests that dexamphetamine and guanethidine act at the same site. There are reasons for thinking that this site may be the noradrenaline store at sympathetic nerve endings.

The suggestion that dexamphetamine owes its sympathomimetic action to the liberation of noradrenaline from a store was first made by Burn & Rand (1958). They based this suggestion on the failure of dexamphetamine to have its usual sympathomimetic action after treatment with reserpine, when the stores of noradrenaline were depleted. The infusion of noradrenaline into cats treated with reserpine increased the store of noradrenaline and restored the responses to dexamphetamine. Recently Schümann & Philippu (1962) showed that amphetamine displaced noradrenaline from storage granules isolated from the adrenal medulla.

Guanethidine has sympathomimetic actions, and these actions resemble those of amphetamine, because both depend on the store of noradrenaline. Thus Wylie (1961) found that guanethidine in the cat caused a pressor response, which he concluded was due to release of catechol amines since it was potentiated by pyrogallol which inhibits O-methyl transferase. Gillis & Nash (1961) showed that guanethidine had a pressor action in rats, and that this was decreased by pretreatment with reserpine and restored by a noradrenaline infusion. The pressor effect was abolished by tolazoline and phenoxybenzamine. In spinal cats (Bartlet, 1962) the pressor response to guanethidine exhibited a marked tachyphylaxis, and in this respect too guanethidine resembles dexamphetamine. We have shown (unpublished observations) that there is a cross-tachyphylaxis between the pressor actions of dexamphetamine and guanethidine.

The first evidence for an interaction between guanethidine and amphetamine came from the work of Maxwell, Mull & Plummer (1959), whose observations were extended by Maxwell, Plummer, Povalski & Schneider (1960). They found that the prior injection of large doses of guanethidine into dogs abolished the pressor response to amphetamine. In our experience smaller doses of guanethidine, just sufficient to abolish the response to sympathetic nerve stimulation, reduced but did not abolish the action of dexamphetamine.

There is a further resemblance between dexamphetamine and guanethidine. The most conspicuous property of guanethidine is its blockade of sympathetic nerve function, and this is the basis of its therapeutic use in relieving hypertension. Dexamphetamine also shares this property, although it is better known for its sympathomimetic action. We have shown here that dexamphetamine blocked the response of the nictitating membrane to nerve stimulation, but did not block the response to noradrenaline. Dexamphetamine blocked the response of the vas deferens to hypogastric nerve stimulation. The effect of dexamphetamine in blocking the response of the isolated rabbit ileum to nerve stimulation was first demonstrated by Åström (1949); this observation was confirmed by Day (1962) and in the present paper.

The antagonism by dexamphetamine of the sympathetic nerve block by guanethidine fulfils the conditions of competitive antagonism as outlined by Arunlakshana & Schild (1959). The assumption made in the derivation of their equation,  $pA_2 - pA_{10} = 0.95$ , is that the agonist and the antagonist are acting on the same receptor. The  $pA_2$  and  $pA_{10}$  values for the agonist (guanethidine) and the antagonist (dexamphetamine) in our experiments yield a value (1.02) in close agreement with the theoretical value of 0.95. This is evidence that guanethidine and dexamphetamine are acting on the same "receptor." If we consider what is known of the way in which guanethidine and dexamphetamine act, then the store of noradrenaline is a candidate for the role of "receptor" in this particular drug interaction. Perhaps both guanethidine and dexamphetamine can become attached to the store of noradrenaline.

In the experiments of Axelrod, Whitby & Hertting (1961) amphetamine reduced the uptake of [<sup>3</sup>H]-noradrenaline and depleted the tissue content of noradrenaline in heart and spleen. Dengler, Spiegel & Titus (1961) also found that amphetamine reduced the uptake and binding of radio-labelled noradrenaline in heart and spleen, and that guanethidine had the same actions. Similar findings for guanethidine were obtained by Hertting, Axelrod & Patrick (1962). The depletion of tissue noradrenaline by guanethidine was demonstrated by Shepherd & Zimmermann (1959) and by Cass *et al.* (1960); Sanan & Vogt (1962) showed that both guanethidine and amphetamine reduced the noradrenaline content of sympathetic ganglia. In the experiments reported here dexamphetamine did not affect the noradrenaline depletion due to guanethidine. Although we think that it is the interaction of dexamphetamine and guanethidine at the noradrenaline storage site which is responsible for the restoration of sympathetic nerve function, this interaction is not directly related to the actual content of noradrenaline in the store. Evidence that the sympathetic nerve block by guanethidine is not directly dependent on noradrenaline depletion

comes from the work of Cass & Spriggs (1961), Sanan & Vogt (1962) and Day & Rand (1962).

We have already proposed another way in which the interaction between dexamphetamine and guanethidine can be considered (Day, 1962; Day & Rand, 1962). Guanethidine has weak sympathomimetic actions and strong sympathetic nerve blocking activity. Dexamphetamine has strong sympathomimetic actions and weak sympathetic nerve blocking activity. As far as the sympathetic nerve blocking activities are concerned, guanethidine can be considered as a strong agonist, in Stephenson's (1956) terminology, and dexamphetamine as a weak agonist. The resultant action of both drugs given together is a smaller effect than of the strong agonist given alone, the resultant being a partial restoration of sympathetic nerve function.

This work was carried out by M. D. Day while he was in receipt of a scholarship from the Medical Research Council. We are indebted to our colleagues T. L. B. Spriggs for spectrofluorometric estimations of tissue noradrenaline and Professor G. A. H. Buttle and A. W. Cuthbert for suggesting some of the experiments.

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