Br. J. Pharmac. (1969), 35, 563–572.

Mechanism of the antagonism between guanethidine and dexamphetamine

J. F. GERKENS, M. W. McCULLOCH AND J. WILSON*

Department of Pharmacology, Melbourne University, Parkville, 3052, Victoria, Australia.

1. The effects of dexamphetamine were studied on the responses of rabbit ileum, rabbit ear artery and sheep spleen to sympathetic nerve stimulation after exposure to guanethidine and in the absence of guanethidine.

2. In the absence of guanethidine, dexamphetamine enhanced the responses to sympathetic stimulation and, in the spleen, this was shown to be due to an increase in noradrenaline output. However, the increase in these responses was much less than the increase obtained in preparations treated with guanethidine.

3. Cocaine, in a concentration which produced the same effect on noradrenaline uptake as the concentration of dexamphetamine used, was also effective in reversing the adrenergic neurone blocking actions of guanethidine.

4. It is suggested that the antagonism between dexamphetamine and guanethidine is due to a reduction in the uptake of guanethidine by the nerve endings rather than to interaction of the two drugs at the receptor site for the adrenergic neurone blocking action of guanethidine.

Soon after the introduction of bretylium and guanethidine into clinical use as antihypertensive agents, it was noticed that their hypotensive action was reduced by simultaneous treatment with amphetamine (Wilson & Long, 1960; Laurence & Rosenheim, 1960). Day & Rand (1962) showed that dexamphetamine prevented or reversed the adrenergic neurone blocking action of guanethidine in anaesthetized cats and dogs. Further experiments led Day & Rand (1963) to suggest that the antagonism between guanethidine and dexamphetamine was competitive and that dexamphetamine was combining with the receptor concerned with the blocking action of guanethidine at sympathetic nerve endings.

The present authors, using innervated ileum preparations, found that the concentrations of dexamphetamine required to reverse the action of guanethidine also potentiated responses to submaximal sympathetic nerve stimulation. The possibility that dexamphetamine might be potentiating a residual amount of sympathetic transmitter released during nerve stimulation after blockade by guanethidine, led to a closer examination of the interaction between the two drugs.

* Present address: Department of Pharmacology and Therapeutics, St. Thomas's Hospital Medical School, London S.E.1.

Methods

Rabbit ileum

Segments of rabbit ileum with their sympathetic nerves were prepared by the method of Finkleman (1930). A length of ileum 2–3 cm long was set up in a 50 ml. bath containing McEwen's (1956) solution at 37° C, and movements of the ileum were recorded using an isotonic frontal-writing lever. The periarterial nerves were stimulated with bipolar platinum electrodes at frequencies of from 2/sec to 20/sec using 1 msec pulses at supramaximal voltage for 30 sec every 4 min.

Rabbit ear artery

The preparation described by De la Lande & Rand (1965) was used. Rabbits weighing 2–3 kg were killed by a blow on the back of the neck. The skin was removed from the ear in the region of the central artery and the artery was dissected free from the surrounding tissues. The artery was cannulated with fine polythene tubing and a length of 3–4 cm was excised. The artery segment was then perfused with McEwen's solution at 37° C and the reservoir of McEwen's solution was gassed with 5% carbon dioxide in oxygen. The flow of the perfusion fluid was maintained with a roller pump at a rate of 5 ml./min. The perfusion pressure was measured with a pressure transducer and recorded on an Offner Dynograph. The periarterial sympathetic nerves were stimulated by means of bipolar platinum ring electrodes placed on the upper end of the vessel. The preparation was stimulated at rates of 2–10 pulses/sec for 10 sec every 2 min, using 1 msec pulses and supramaximal voltage. Drugs were injected into the perfusion fluid just before it reached the cannula, by means of a Palmer slow injection apparatus. The drugs were dissolved in McEwen's solution and injected at a rate of 0.1 to 0.2 ml./min to avoid injection artefacts. Drug concentrations were expressed as μg/ml. of the perfusion fluid.

Sheep spleen

Spleens were collected from freshly killed sheep and stored in McEwen's solution from the time of their removal until they were set up for perfusion about 1 hr later. Glass cannulae were inserted into the splenic artery and splenic vein and the two main branches of the nerve were dissected free from the artery and placed over bipolar platinum stimulating electrodes. The spleen was perfused with McEwen's solution previously warmed to 37° C and gassed with 5% carbon dioxide in oxygen. Perfusion was maintained at a rate of 40 ml./min by means of a constant volume roller pump. During perfusion the preparation was immersed in liquid paraffin maintained at 37° C. The splenic nerve was stimulated for 1 min every 25 min using 1 msec pulses at a rate of 5 to 20/sec and supramaximal voltage. The venous effluent was collected during the last 30 sec of stimulation. Ascorbic acid (1 mg/ml.) was added to the sample which was then centrifuged and stored in ice. The samples were assayed for catecholamines using the modified pithed rat blood pressure method described by Haefely, Hürliman & Thoenen (1965).

The total catecholamine content of each sample was expressed in terms of noradrenaline. The effects of drugs on the uptake of noradrenaline were examined in the following way. Noradrenaline (0.1 or 0.2 μg/ml.) was infused into the perfusion fluid for 5 min in every 25 min. The venous effluent was collected for the last 30 sec of the infusion and assayed for noradrenaline as before. The fall in the concentration of noradrenaline in the perfusion fluid between entering and leaving the spleen was taken as the percentage uptake of noradrenaline.

Results

Rabbit ileum

Stimulation of the periarterial sympathetic nerves to isolated segments of rabbit ileum at a rate of 20 pulses per second caused a 90–100% inhibition in the pendular movements. The addition of guanethidine (0.2 μg/ml.) to the organ bath produced a gradual reduction in these responses until, after about 30 min, sympathetic stimulation produced only 10 to 30% inhibition. Dexamphetamine (0.2 μg/ml.) added to the bath at this stage caused an almost complete reversal of the blockade (Fig. 1). However, in a preparation which had not been treated with guanethidine, stimulated at a low frequency which produced only 10 to 30% inhibition, dexamphetamine also produced a marked increase in the size of the responses. Thus Fig. 1 shows an experiment in which dexamphetamine (0.2 μg/ml.) increased the responses of the rabbit ileum to stimulation at a frequency of 3/sec from 20% inhibition to an almost complete inhibition. In order to determine whether the apparent antagonism between dexamphetamine and guanethidine was due solely to the enhancement of responses to sympathetic nerve stimulation by dexamphetamine, experiments were carried out as follows. The frequency of stimulation was adjusted in each preparation so that the ileum responded with an inhibition of about 20% either in the absence of guanethidine or after about 30 min exposure to guanethidine (0.2 μg/ml.). The effects of dexamphetamine in concentrations of 0.05, 0.1 and 0.2 μg/ml. were then determined on guanethidine-treated and untreated segments of ileum, each experiment being repeated four to six times. The results of the experiments are

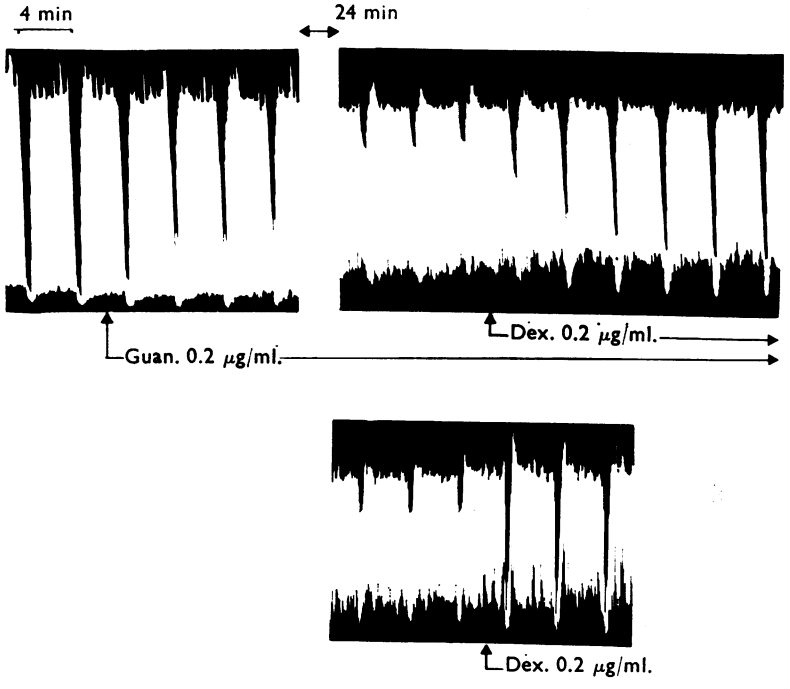


FIG. 1. Rabbit ileum. The sympathetic nerves were stimulated for 30 sec every 4 min using 1 msec pulses at a frequency of 20/sec in the upper trace and 3/sec in the lower trace. The records show the effects of dexamphetamine (0.2 μg/ml.) after exposure to 0.2 μg/ml. of guanethidine (upper trace) and in the absence of guanethidine (lower trace).

shown in Fig. 2. With all three concentrations of dexamphetamine the mean increase in inhibition was greater in the presence of guanethidine than in untreated preparations. The difference between the mean increase in response in treated and untreated preparations was significant with a concentration of dexamphetamine of 0.2 μg/ml. ($0.02>P>0.01$). With lower concentrations of dexamphetamine the difference was not statistically significant (0.1 μg/ml., $0.1>P>0.05$; 0.05 μg/ml., $0.3>P>0.2$).

Rabbit ear artery

Guanethidine (0.5 μg/ml.) produced a gradual reduction in the responses of the artery preparation to stimulation of the sympathetic nerves and after 50 min, these responses were virtually abolished (Fig. 3). After a further 40 min perfusion without guanethidine, there was no recovery of the responses to sympathetic nerve

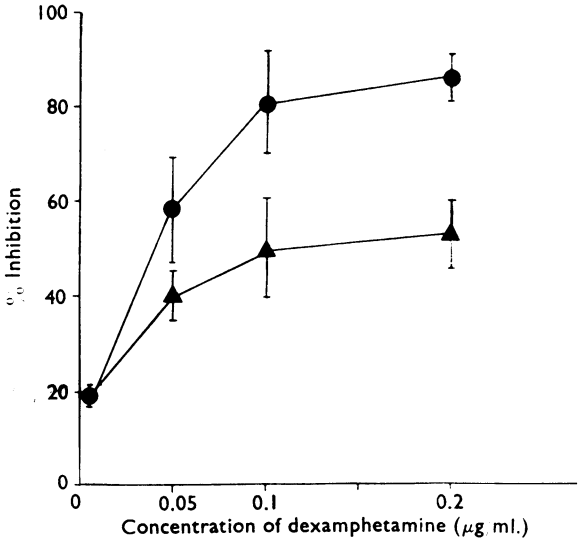


FIG. 2. Rabbit ileum. The sympathetic nerves were stimulated for 30 sec every 4 min using 1 msec pulses. The frequency was adjusted to produce approximately 20% inhibition of the pendular movements either in the absence of guanethidine or after approximately 30 min exposure to guanethidine (0.2 μg/ml.). The graphs show the effects of dexamphetamine (0.05, 0.1 and 0.2 μg/ml.) on the responses to sympathetic nerve stimulation in the presence of guanethidine (●) and in untreated preparations (▲). Each point is the mean of from four to six observations, the vertical bars being the standard errors.

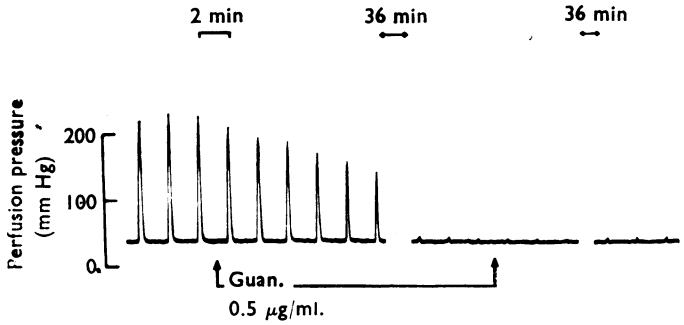


FIG. 3. Isolated rabbit ear artery. The periarterial sympathetic nerves were stimulated for 10 sec every 2 min using 1 msec pulses at a frequency of 20/sec. The record shows blockade of the responses by guanethidine (0.5 μg/ml.). Forty minutes after the infusion of guanethidine was stopped there was virtually no recovery of the responses.

stimulation. The addition of dexamphetamine (0.2 μg/ml.) or cocaine (2 μg/ml.) to the perfusion fluid, however, produced a complete reversal of the blockade (Fig. 4). The possibility was considered that cocaine and dexamphetamine might be simply potentiating a small amount of noradrenaline released by nerve stimulation after blockade by guanethidine. The small responses which remained after exposure to guanethidine were therefore mimicked in untreated preparations by stimulating at low frequencies. In these experiments dexamphetamine (0.2 μg/ml.) and cocaine (2 μg/ml.) produced only a slight increase in the responses (Fig. 5).

Sheep spleen

When the splenic nerve was stimulated there was an increase in perfusion pressure in the spleen and noradrenaline appeared in the venous effluent. In the presence of guanethidine (0.2 μg/ml.) there was a considerable reduction in both the rise in perfusion pressure and the noradrenaline output (Fig. 6a). These were partly

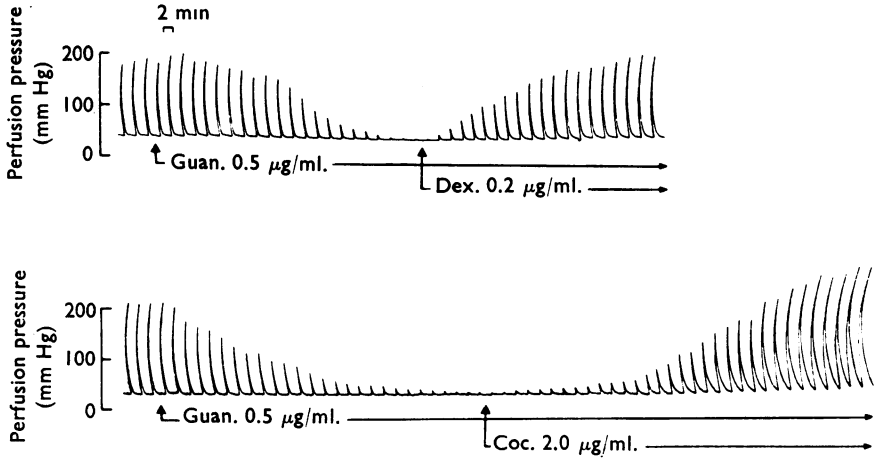
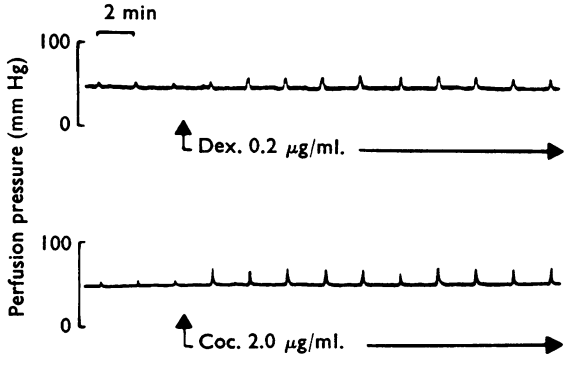


FIG. 4. Isolated rabbit ear artery. The periarterial sympathetic nerves were stimulated for 10 sec every 2 min using 1 msec pulses at a frequency of 15/sec. The records show the effects of dexamphetamine (0.2 μg/ml.) and cocaine (2 μg/ml.) on the adrenergic neurone blockade produced by guanethidine (0.5 μg/ml.).

FIG. 5. Isolated rabbit ear artery. The upper record shows the effect of dexamphetamine (0.2 μg/ml.) on the responses to periarterial sympathetic nerve stimulation for 10 sec every 2 min using 1 msec pulses at a rate of 0.6/sec. The lower record shows the effect of cocaine (2 μg/ml.) on the responses to periarterial stimulation for 4 sec every 2 min using 1 msec pulses at a rate of 2/sec.



restored by the addition of dexamphetamine (0.2 μg/ml.) to the perfusion fluid. The combined results of four such experiments are shown in Fig. 6b, from which it can be seen that 75 min after the addition of dexamphetamine the noradrenaline output was about 20 times greater than immediately before its addition. Dexamphetamine also increased the noradrenaline output in response to submaximal sympathetic nerve stimulation in the absence of guanethidine. The effect of dexamphetamine (0.2 μg/ml.) on the output of noradrenaline during stimulation of the splenic nerve at a rate of 5 pulses/sec in four experiments is shown in Fig. 7. This frequency produced a response which was similar in size to that obtained with maximal stimulation after exposure to guanethidine in the above experiments. The increase in noradrenaline output was about 3-fold, and was therefore much less marked than in the guanethidine-treated preparations. It seemed likely that the increased concentration of noradrenaline in the venous effluent was due to a reduction in its re-uptake into the sympathetic nerve endings. Noradrenaline was there-

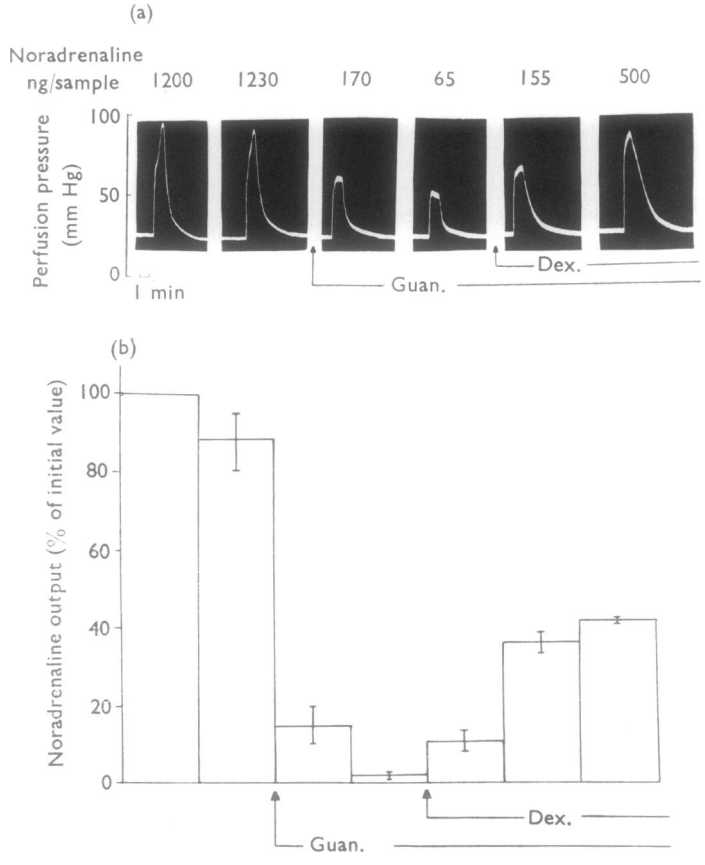


FIG. 6. Isolated perfused sheep spleen. (a): The splenic nerves were stimulated for 60 sec every 25 min using 1 msec pulses at a rate of 20/sec. The venous effluent was collected during the last 30 sec of each period of stimulation and the figures shown above the record give the total noradrenaline (ng) in the samples collected. The record shows the effects of guanethidine (0.2 μg/ml.) and dexamphetamine (0.2 μg/ml.) on the preparation. (b): Histogram showing the combined results of four experiments identical to that shown in (a). The noradrenaline output is expressed as a percentage of the noradrenaline content of the sample collected during the initial response and the standard errors are shown by the vertical bars.

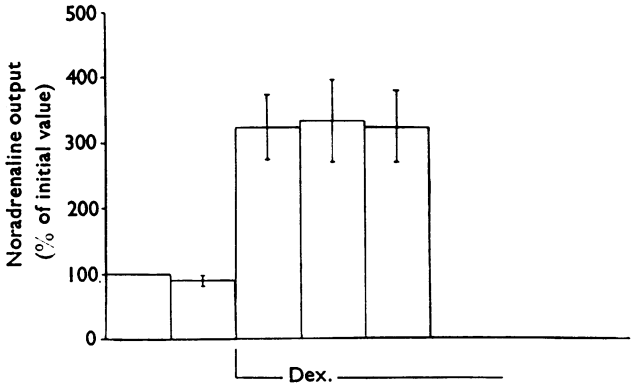


FIG. 7. Isolated perfused sheep spleen. The splenic nerves were stimulated for 60 sec every 25 min using 1 msec pulses at a rate of 5/sec. NA output is the amount of noradrenaline appearing in the venous effluent during the last 30 sec of each period of stimulation and is expressed as a percentage of the amount in the sample collected during the first period of stimulation. The histogram shows the effects of dexamphetamine (0.2 μg/ml.) on the noradrenaline output. The mean values from four experiments are shown and the vertical bars represent the standard errors.

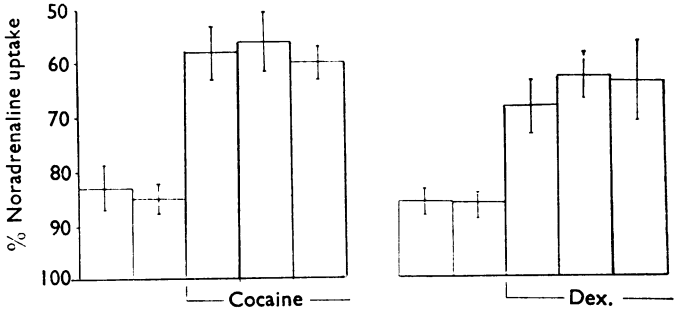


FIG. 8. Isolated perfused sheep spleen. Percentage noradrenaline uptake was measured by infusing noradrenaline (0.1 μg/ml.) into the spleen and measuring the concentration of noradrenaline appearing in the venous effluent. Noradrenaline was infused for 5 min every 25 min. The histograms show the effects of cocaine (0.5 μg/ml.) and dexamphetamine (0.2 μg/ml.) on the noradrenaline uptake. Mean values are shown from three experiments using each drug and the vertical bars show the standard errors.

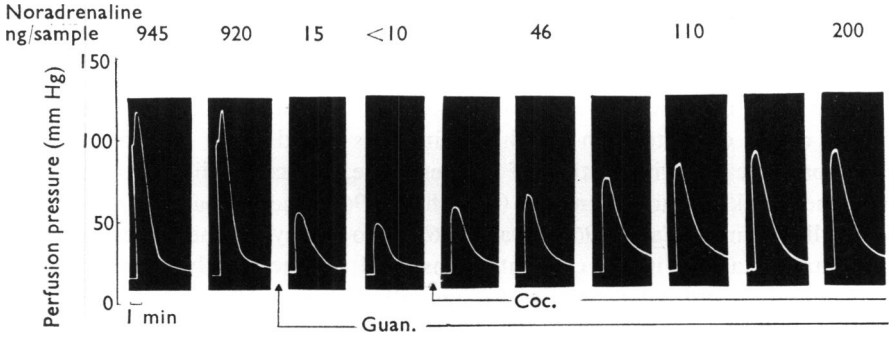


FIG. 9. Isolated perfused sheep spleen. The splenic nerves were stimulated for 60 sec every 25 min using 1 msec pulses at a rate of 20/sec. The venous effluent was collected during the last 30 sec of each period of stimulation and the figures shown give the total noradrenaline in the samples collected. The record shows the effects of guanethidine (0.2 μg/ml.) and cocaine (0.5 μg/ml.).

fore infused at intervals into the spleen and its uptake was studied before and after the addition of dexamphetamine. The mean uptake of an infusion of noradrenaline (0.1 μg/ml.) during perfusion through the spleen was reduced from $86.5 \pm 3\%$ to $64 \pm 7.4\%$ when dexamphetamine (0.2 μg/ml.) was added to the perfusion fluid (Fig. 8). Cocaine (0.5 μg/ml.) was found to have a similar effect on uptake of noradrenaline to 0.2 μg/ml. of dexamphetamine (Fig. 8). Perfusion of the preparation with cocaine (0.5 μg/ml.) reduced noradrenaline uptake from $85 \pm 2.8\%$ to $60 \pm 3\%$. Figure 9 shows that cocaine (0.5 μg/ml.) was also effective in reversing the blocking action of guanethidine although the reversal was more gradual than that produced by dexamphetamine.

Discussion

Day & Rand (1963) made a detailed study of the antagonism between dexamphetamine and guanethidine using the Finkleman (1930) preparation of rabbit ileum. As a result of their experiments and from a consideration of similarities in some of the actions of the two drugs, they suggested that dexamphetamine was competing with guanethidine for the site of its blocking action at the noradrenaline store. In the present experiments using the same preparation it was found that dexamphetamine, in the concentration necessary to reverse the blocking action of guanethidine, enhanced the responses to sympathetic nerve stimulation. This enhancement was seen most clearly on responses to low frequency stimulation. When the frequency was adjusted to produce approximately 20% inhibition, the effect of dexamphetamine on the responses ranged from a slight increase to the production of complete inhibition. A response giving 20% inhibition was selected to match the response to maximal stimulation obtained after 30 min exposure to guanethidine (0.2 μg/ml.). After repeating the experiments a number of times it was found that the increase in the responses was considerably greater in the preparations treated with guanethidine than in untreated preparations.

Dexamphetamine has been shown to block the uptake of both noradrenaline (Iversen, 1964) and guanethidine (Chang, Costa & Brodie, 1965) into the sympathetic nerve endings. Blockade of the re-uptake of noradrenaline could account for the increase in the responses to sympathetic stimulation of the preparations not treated with guanethidine but could not alone account for the antagonism between dexamphetamine and guanethidine. However, if it is assumed that the guanethidine in the sympathetic nerves is in equilibrium with that in the bath fluid, blockade of the uptake of guanethidine might be expected to result in a net movement of guanethidine out of the nerve. This suggestion was supported by the experiments with the rabbit ear artery, in which cocaine was found to be effective in reversing the sympathetic blocking action of guanethidine. Cocaine, like dexamphetamine, blocks the uptake of noradrenaline (Muscholl, 1961) and probably also of guanethidine (Callingham & Cass, 1962 ; Day, 1962) into the sympathetic nerves. Cocaine and dexamphetamine in the concentrations used produced only a small increase in the responses of untreated arteries to sympathetic nerve stimulation. The antagonism between these drugs and guanethidine therefore appears to be due to the removal of guanethidine from the site of its blocking action.

A more detailed examination of the antagonism between dexamphetamine and guanethidine was carried out using the isolated perfused sheep spleen in which noradrenaline release and uptake could be measured. Maximal stimulation of the

spleen produced a rise in perfusion pressure and a sharp increase in the noradrenaline content of the venous effluent. The rises in perfusion pressure and noradrenaline overflow were greatly reduced by guanethidine and subsequently partially restored by dexamphetamine. As was found with rabbit ileum and ear artery preparations, the responses of spleens not treated with guanethidine to submaximal sympathetic nerve stimulation were also enhanced after adding dexamphetamine. This increase was, however, much smaller than the enhancement in responses produced by dexamphetamine in guanethidine-treated spleens. Experiments in which noradrenaline uptake by the spleen was measured showed that dexamphetamine impaired uptake of noradrenaline. This inhibition of noradrenaline uptake was sufficient to account for the increase in noradrenaline overflow in spleens not treated with guanethidine. When the responses of the spleen had been partly blocked by guanethidine, dexamphetamine increased noradrenaline overflow to a much greater extent. A concentration of cocaine was found which reduced noradrenaline uptake by a similar amount to the concentration of dexamphetamine used in the above experiments. This concentration of cocaine was also found to be effective in reversing adrenergic neurone blockade by guanethidine in the spleen.

There is now a considerable body of evidence to suggest that guanethidine is taken up into the sympathetic nerve endings by the uptake process for noradrenaline (Chang, Costa & Brodie, 1965 ; Obianwu, Stitzel & Lundborg, 1968). It therefore seems likely that the reversal of guanethidine by dexamphetamine is due to competition with guanethidine for the uptake process rather than for the site of the adrenergic neurone blocking action.

Sympathetic nerves exposed to guanethidine have been shown to release guanethidine when stimulated (Boullin, Costa & Brodie, 1966). The action of guanethidine is normally very persistent, so most of the drug released in this way would presumably be taken up again into the sympathetic nerves. In the presence of sufficient dexamphetamine to impair its re-uptake, however, the guanethidine would be lost from the sympathetic nerve endings and the blockade reversed. Dexamphetamine has a prolonged sympathomimetic effect which is reduced but not abolished in the presence of a full adrenergic neurone blocking concentration of guanethidine. Because this action is due to the release of noradrenaline from neuronal stores, it is possible that guanethidine accumulated at this site would be released simultaneously. This would account for the observation that reversal of the blocking action of guanethidine was rather more rapid with dexamphetamine than with cocaine.

An affinity for the noradrenaline storage site could well explain the accumulation of guanethidine in the sympathetic nerve endings. The mechanism by which this high local concentration of guanethidine blocks sympathetic nerve transmission is, however, still uncertain. Rand & Wilson (1967) examined a number of compounds related to guanethidine and, on the basis of structure-activity studies, suggested that guanethidine interacted with a cholinergic receptor involved with transmission at sympathetic nerve endings (Burn & Rand, 1959). It was rather difficult to reconcile this view with the hypothesis of Day & Rand (1963) that dexamphetamine acts at the site of the blocking action of guanethidine for it seems unlikely that dexamphetamine in low concentrations would act on cholinergic receptors. However, the present work is not incompatible with the mode of action of guanethidine proposed by Rand & Wilson (1967). It suggests that the antagonism between dexamphetamine and guanethidine is due primarily to the effect of dexamphetamine on the

uptake of guanethidine into the nerve endings rather than antagonism at the receptor responsible for the blocking action of guanethidine.

The skilful technical assistance of Miss S. Matrai is gratefully acknowledged. We are also indebted to Professor M. J. Rand and Professor R. S. Stacey for helpful discussion of the manuscript.

REFERENCES

BOULLIN, D. J., COSTA, E. & BRODIE, B. B. (1966). Discharge of tritium-labelled guanethidine by sympathetic nerve stimulation as evidence that guanethidine is a false transmitter. *Life Sci., Oxford*, **5**, 803–808.

BURN, J. H. & RAND, M. J. (1959). Sympathetic postganglionic mechanism. *Nature, Lond.*, **184**, 163–165.

CALLINGHAM, B. A. & CASS, R. (1962). The effects of bretylium and cocaine on noradrenaline depletion. *J. Pharm. Pharmac.*, **14**, 385–389.

CHANG, C. C., COSTA, E. & BRODIE, B. B. (1965). Interaction of guanethidine with adrenergic neurones. *J. Pharmac. exp. Ther.*, **147**, 303–312.

DAY, M. D. (1962). Effect of sympathomimetic amines on the blocking action of guanethidine, bretylium and xylocholine. *Br. J. Pharmac. Chemother.*, **18**, 421–439.

DAY, M. D. & RAND, M. J. (1962). Antagonism of guanethidine by dexamphetamine and other related sympathomimetic amines. *J. Pharm. Pharmac.*, **14**, 541–549.

DAY, M. D. & RAND, M. J. (1963). Evidence for a competitive antagonism of guanethidine by dexamphetamine. *Br. J. Pharmac. Chemother.*, **20**, 17–28.

DE LA LANDE, I. S. & RAND, M. J. (1965). A simple isolated nerve-blood vessel preparation. *Aust. J. exp. Biol. med. Sci.*, **43**, 639–656.

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

HAEFELY, W., HÜRLIMANN, A. & THOENEN, H. (1965). Relation between the rate of stimulation and the quantity of noradrenaline liberated from sympathetic nerve endings in the isolated perfused spleen of the cat. *J. Physiol., Lond.*, **181**, 48–58.

IVERSEN, L. L. (1964). The inhibition of noradrenaline uptake by sympathomimetic amines. *J. Pharm. Pharmac.*, **16**, 435–437.

LAURENCE, D. R. & ROSENHEIM, M. L. (1960). *Ciba Foundation Symposium on Adrenergic Mechanisms*, pp. 201–208. London: J. and A. Churchill Ltd.

MCEWEN, L. M. (1956). The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol., Lond.*, **131**, 678–689.

MUSCHOLL, E. (1961). Effect of cocaine and related drugs on the uptake of noradrenaline by heart and spleen. *Br. J. Pharmac. Chemother.*, **16**, 352–359.

OBIANWU, H. O., STITZEL, R. & LUNDBORG, P. (1968). Subcellular distribution of (^{3}H) amphetamine and (^{3}H) guanethidine and their interaction with adrenergic neurones. *J. Pharm. Pharmac.*, **20**, 585–594.

RAND, M. J. & WILSON, J. (1967). Receptor site of adrenergic neuron blocking drugs. *Circulation Res.*, **20**, Suppl. 3, 89–99.

WILSON, R. & LONG, C. (1960). Action of bretylium antagonised by amphetamine. *Lancet*, **2**, 262.

(*Received December* 11, 1968)