Br. J. Pharmac. (1974), **51**, 237-247

THE RELATION BETWEEN THE ADRENERGIC NEURONE-BLOCKING AND NORADRENALINE-DEPLETING ACTIONS OF SOME GUANIDINE DERIVATIVES

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1 The effects of some guanidine derivatives, (−)-*N*-(1-phenylethyl) guanidine (PEG), guanethidine and debrisoquine have been investigated on the content and subcellular distribution of noradrenaline in cat spleen and on the overflow of noradrenaline from this organ during stimulation of the splenic nerve.

2 PEG and guanethidine, at a dose of 5 mg/kg, produced adrenergic neurone blockade within 15 min and the same dose of debrisoquine produced blockade within 30 minutes.

3 All three compounds produced a decrease of similar magnitude in the noradrenaline content of the high-speed particulate (P_2) and supernatant (S) fractions of splenic homogenates; these actions were temporally correlated with the adrenergic neurone-blocking action of the compounds.

4 PEG did not produce any further decrease in the noradrenaline content of the subcellular fractions at times up to 18 h after its administration; adrenergic neurone blockade was maintained throughout this period but had disappeared after 7 days when the noradrenaline content of the subcellular fractions was restored to control levels.

5 Guanethidine, in contrast, caused a marked progressive loss of the transmitter from all subcellular fractions—an effect which was maximal 18 h after its administration but continued, as did the adrenergic neurone-blocking action, for at least 72 hours. This additional loss of noradrenaline, over and above that seen after 15 min, is unlikely to be connected with the adrenergic neurone-blocking action of the drug.

6 Dexamphetamine both prevented and antagonized the neurone blockade and the subcellular noradrenaline-depleting action of PEG and guanethidine. The restoration of nerve function after administration of dexamphetamine to animals pretreated with 5 mg/kg of either of these compounds was temporally correlated with a selective repletion of the noradrenaline content of the supernatant fraction of the spleen.

7 Larger doses (15 mg/kg) of PEG or guanethidine produced a selective depletion of noradrenaline in only the supernatant fraction of the spleen. This depletion was temporally correlated with the adrenergic neurone-blocking action of these compounds. The lack of effect of the compounds at this dose level on the noradrenaline contained in the P_2 fraction may be due to 'stabilization' of the store of noradrenaline *in vivo* which gives rise to this fraction on homogenization.

8 It is suggested that the guanidine-type adrenergic neurone-blocking agents displace the noradrenaline which is readily available for release by nerve impulses, and that it is this action that may account for their sympathomimetic properties.

9 The ability of these guanidines to impair the release of noradrenaline by nerve impulses could occur because whilst they are present within the neurone the 'nerve-releasable store', which may in these experiments appear in the supernatant fraction after homogenization, may be unable to refill with transmitter.

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Introduction

Previous experiments with bretylium and debrisoquine (Abbs & Robertson, 1970; Robertson & Abbs, 1971; Abbs & Pycock, 1973) support the hypothesis (Abbs, 1966; Carlsson, 1966) that adrenergic neurone-blocking agents may act by a selective displacement of a small 'store' of noradrenaline which is essential for the proper functioning of adrenergic nerves.

The purpose of this study was to extend these observations by using the two guanidine derivatives, (−)-*N*-(1-phenylethyl) guanidine sulphate (PEG) and guanethidine, and to compare some of their actions with debrisoquine.

Both PEG and guanethidine prevent the release of noradrenaline from adrenergic nerve terminals in response to electrical stimulation but have contrasting effects on the tissue content of the adrenergic neurotransmitter. PEG does not reduce endogenous noradrenaline levels, even in doses in excess of those required to produce adrenergic neurone blockade (Fielden, Green & Willey, 1965), whereas even with small doses of guanethidine there is a marked loss of noradrenaline from adrenergically-innervated tissues (Cass, Kuntzman & Brodie, 1960). However, with guanethidine there is a clear dissociation between its noradrenaline-depleting and adrenergic neurone-blocking actions (Cass & Spriggs, 1961; Gaffney, Chidsey & Braunwald, 1963; Spriggs, 1966).

It was thus decided to partition the 'stores' of noradrenaline by subcellular fractionation techniques and to investigate whether the two guanidines produced any significant depletion of transmitter from the subdivided 'stores' at times when their adrenergic neurone-blocking action was apparent.

Methods

Preparation of animals

Male, female or neutered adult cats were used. Anaesthesia was induced with ether and maintained with chloralose (80 mg/kg, i.v.). The animals were then prepared either for removal of spleens or for collection of splenic venous blood as described by Abbs & Robertson (1970). Blood pressure was recorded routinely from the left femoral artery.

Animals in acute, subacute (18 h) or chronic (7 days) experiments received an intravenous injection of either the drugs under test or 0.9% w/v NaCl solution (saline).

Details of doses and time schedules are given in the appropriate section of the results.

Collection and treatment of blood samples for determination of noradrenaline levels in plasma

Splenic venous blood was collected before, during, and for 30 s after stimulation of splenic nerves as described by Abbs & Robertson (1970). This technique allowed the determination of 'resting' plasma levels of noradrenaline, plasma levels after drug treatment and the determination of the noradrenaline overflowing into plasma of splenic venous blood in response to nerve stimulation.

Plasma from each sample was separated, deproteinized with perchloric acid, and stored overnight at −20° C until required for purification and assay.

Subcellular fractionation of the spleen

Spleens were removed and fractionated as described by Abbs & Robertson (1970) to produce the P_1 (cell debris and mitochondria), P_2 (microsomes), S (supernatant layer) and T (unfractionated homogenate) fractions. The fractions were then deproteinized with perchloric acid and stored overnight at −20° C until required for purification and assay.

Separation and assay of noradrenaline and adrenaline

Noradrenaline and adrenaline in deproteinized plasma or spleen extracts were purified by ion-exchange chromatography and were assayed fluorimetrically by the method of Abbs & Robertson (1970). None of the drugs used interfered with the separation, recovery and assay of noradrenaline and adrenaline.

Estimation of deoxyribonucleic acid-phosphorus (DNA-P) content of cat spleen

Deoxyribose attached to purine bases in the deoxyribonucleic acid molecule was extracted from the residue remaining after noradrenaline and adrenaline had been extracted from the T fraction. DNA-P was assayed as described by Dearnaley & Geffen (1966). None of the drugs used interfered with the estimation.

Drugs

PEG, guanethidine, debrisoquine and dexamphetamine were used in the form of sulphates. The

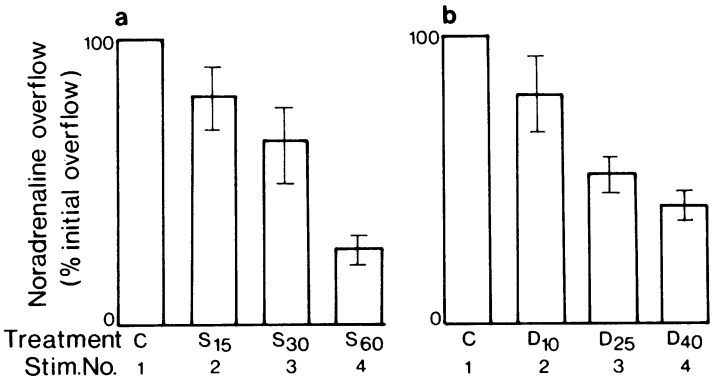

Fig. 1 Overflow of noradrenaline during splenic nerve stimulation. Results are means with s.e. mean. (a) Saline (S; 1 ml/kg) was injected 15 min after the first stimulation; subscripts after S show time (min) after injection of saline; (b) Dexamphetamine (D; 2.5 mg/kg) was injected into cats 15 min after the initial stimulation; subscripts after D indicate time (min) after injection of dexamphetamine. C, controls. Numbers of observations: in (a) = 7, in (b) = 4.

doses used are expressed in terms of the respective salts. All drugs were dissolved in saline and were administered intravenously. Saline was used alone in control experiments and was also administered intravenously.

Results

All results quoted are uncorrected for recovery. The adrenaline content of spleen was less than 10% of the noradrenaline content and was not considered further. Student's *t* test was used to test for significance of differences between means.

The noradrenaline content of the spleen and of its subcellular fractions was expressed as ng noradrenaline/μmol DNA-P. The sum of the noradrenaline contents of the P_1, P_2 and S fractions was approximately 80% of the experimentally determined total content of noradrenaline (T fraction) both in control experiments and in treated animals. DNA-P contents of spleens from control and treated animals were not significantly different from each other.

Noradrenaline overflow, in response to splenic nerve stimulation, was calculated as described by Abbs & Robertson (1970).

None of the drugs used completely prevented the contraction of the splenic capsule in response to stimulation of the splenic nerve, neither did they significantly affect splenic plasma volumes. Thus when there was a reduction in overflow, after splenic nerve stimulation, to below 5% of the initial overflow, this was interpreted as showing that the release of transmitter from the splenic nerves was impaired.

The noradrenaline overflow varied considerably from cat to cat and therefore an initial overflow was measured in each experiment and subsequent overflows were expressed as a percentage of this initial value.

Control experiments

Plasma noradrenaline. An initial stimulation was made and saline (1 ml/kg) was administered 15 min later. Stimulations 2 and 3 were performed at 15 min intervals thereafter and a fourth stimulation was delivered after a further 30 minutes.

Noradrenaline overflow declined progressively with successive stimulation trains (Figure 1a). This phenomenon has been shown previously by Abbs & Robertson (1970) to be dependent on the number of previous stimulations rather than on the time interval between them.

The noradrenaline concentrations (ng/ml) in resting samples at 15, 30 and 60 min after the injection of saline (1 ml/kg), were (means ± s.e. mean): 0.8 ± 0.2; 0.5 ± 0.1; 0.2 ± 0.1. Numbers of observations were 7, 14 and 14 respectively.

Splenic noradrenaline. The subcellular distribution of noradrenaline in twelve spleens removed at various times, up to 1 h, after the injection of saline (1 ml/kg) is shown in Table 1. Approximately half (53%) of the noradrenaline was recovered from the P_1 fraction; the remainder was

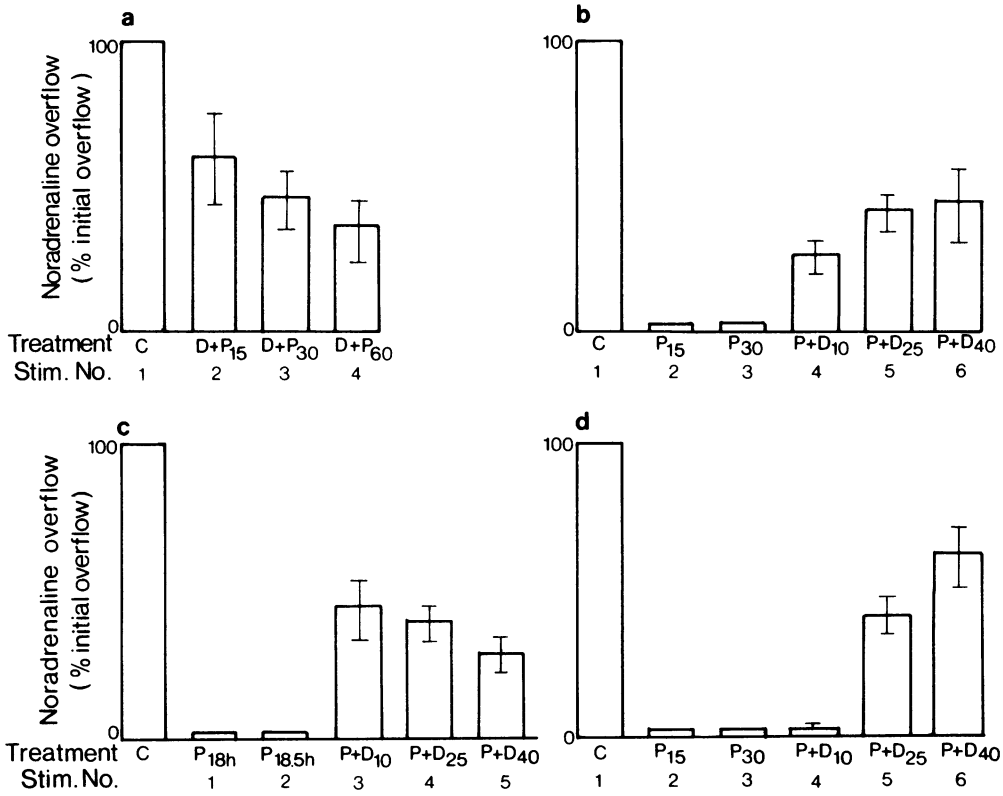

Fig. 2 Interaction of (−)-*N*-(1-phenylethyl)guanidine (PEG) and dexamphetamine on the overflow of noradrenaline during splenic nerve stimulation. Results are means with s.e. mean. (a) Effect of injection of PEG (5 mg/kg) (P) into cats injected 40 min previously with dexamphetamine (2.5 mg/kg) (D); subscripts show time (min) after injection of PEG. (b) Effect of injection of dexamphetamine (2.5 mg/kg) into cats injected 30 min previously with PEG (5 mg/kg); subscripts following P indicate time (min) after injection of PEG; subscripts following P + D indicate time (min) after injection of dexamphetamine into the PEG-pretreated cats. (c) Effect of dexamphetamine (2.5 mg/kg) injected into cats which had been injected with PEG (5 mg/kg) 18 h before the first stimulation; control results (C) are here expressed as the mean of 27 experiments in which the initial stimulation was made before drug treatment; subscripts following P indicate time (h) after injection of PEG; subscripts following P + D indicate time (min) after injection of dexamphetamine into the PEG-pretreated cats. (d) Effect of injection of dexamphetamine (2.5 mg/kg) into cats injected 30 min previously with PEG (15 mg/kg); subscripts following P indicate time (min) after injection of PEG; subscripts following P + D indicate time (min) after injection of dexamphetamine into the PEG-pretreated cats. Numbers of observations: in (a) = 4, (b) = 5, in (c) and (d) = 4.

Table 1 Content and subcellular distribution of noradrenaline in spleens from control and dexamphetamine-treated cats

	Spleen fraction			
	P_1	P_2	*S*	*T*
Treatment	*Noradrenaline content (ng/μmol DNA-P)*			
Saline (*n* = 12)	43 ± 5.1	19 ± 1.3	19 ± 1.5	93 ± 8.7
Dexamphetamine, 10 min (*n* = 4)	34 ± 4.4	15 ± 2.2	16 ± 2.1	72 ± 6.3
Dexamphetamine, 25 min (*n* = 4)	36 ± 2.5	20 ± 2.7	20 ± 2.9	86 ± 6.6
Dexamphetamine, 40 min (*n* = 4)	39 ± 3.5	19 ± 1.5	20 ± 3.1	89 ± 5.0

Dose of dexamphetamine was 2.5 mg/kg. Results quoted are means ± s.e. mean. *n*, number of observations.

divided approximately equally between the P_2 and S fractions.

Dexamphetamine treatment

Plasma noradrenaline. Dexamphetamine (2.5 mg/kg) was without detectable effect on the overflow of noradrenaline consequent upon splenic nerve stimulation (Figure 1b). It did however produce a significant increase ($P < 0.001$) in the noradrenaline concentration (ng/ml) in resting samples of splenic venous blood at 10, 25 and 40 min after its administration. The results (means ± s.e. mean, $n = 4$) were: 7 ± 0.3; 6 ± 0.6; 4 ± 0.9, respectively. There was a marked rise in blood pressure which accompanied these increases in plasma levels of noradrenaline.

Splenic noradrenaline. Cats received an injection of saline (1 ml/kg) followed 30 min later by dexamphetamine (2.5 mg/kg) and spleens were removed 10, 25 or 40 min later. Dexamphetamine had no significant effect on the content and subcellular distribution of noradrenaline in the spleen (Table 1).

(−)-N-(1-phenylethyl)guanidine (PEG) treatment (5 mg/kg)

Plasma noradrenaline. PEG produced a marked rise in blood pressure and rapidly reduced the overflow of noradrenaline to undetectable levels in response to nerve stimulation. This abolition of noradrenaline overflow was evident at 15 min and 18.5 h after injection (Fig. 2, b and c) but had disappeared after 7 days.

The drug also produced a significant elevation ($P < 0.01$) in the resting noradrenaline concentration of splenic venous blood 15 min after administration but this increase was not maintained. The concentration (ng/ml) of noradrenaline in the 15 min resting sample was (mean ± s.e. mean, $n = 5$): 9 ± 1.3.

Splenic noradrenaline. From 15 min to 18 h after the administration of PEG, there were significant decreases of similar magnitude in the noradrenaline contents of both the P_2 and S fractions of cat spleens (Table 2). There were no significant changes in the total noradrenaline content of the spleen at these times; similarly no change was observed in the noradrenaline content of the P_1 fraction.

The decrease in the noradrenaline content of the P_2 and S fractions coincided with the abolition of noradrenaline overflow in response to nerve stimulation.

After 7 days, the noradrenaline content of the P_2 and S fractions had returned to control values (Table 2).

Dexamphetamine treatment in PEG (5 mg/kg)-pretreated animals

Plasma noradrenaline. Administration of dexamphetamine (2.5 mg/kg) to cats pretreated with PEG (5 mg/kg) for either 30 min or for 18.5 h produced a substantial restoration of noradrenaline overflow in response to splenic nerve stimulation (Figure 2, b and c). The mean noradrenaline overflows 10, 25 or 40 min after the administration of dexamphetamine were similar.

Splenic noradrenaline. Cats were pretreated for 30 min with PEG (5 mg/kg) and were then given dexamphetamine (2.5 mg/kg). Spleens were removed 10 or 40 min later. At neither of these times was there a significant depletion of

Table 2 The effect of (−)-*N*-(1-phenylethyl)guanidine (PEG) (5 mg/kg) on the content and subcellular distribution of noradrenaline in cat spleen

	Spleen fraction			
	P_1	P_2	S	T
Treatment	*Noradrenaline content (ng/μmol DNA-P)*			
Saline ($n = 12$)	43 ± 5.1	19 ± 1.3	19 ± 1.5	93 ± 8.7
PEG, 15 min ($n = 7$)	37 ± 4.4	12 ± 2.2*	10 ± 2.0**	76 ± 10.0
PEG, 30 min ($n = 6$)	36 ± 5.1	12 ± 0.8**	10 ± 2.1**	78 ± 10.4
PEG, 60 min ($n = 6$)	39 ± 5.7	12 ± 1.9**	10 ± 1.6**	77 ± 10.0
PEG, 18 h ($n = 4$)	36 ± 8.4	12 ± 2.0*	11 ± 2.1*	73 ± 13.6
PEG, 7 days ($n = 4$)	41 ± 6.0	19 ± 1.8	19 ± 0.8	97 ± 14.1

Results quoted are means ± s.e. mean. *n*, number of observations.
* $P < 0.05$; ** $P < 0.01$.

noradrenaline in the spleen or in its subcellular fractions (Table 3).

Similar experiments were carried out with cats treated 18 h previously with PEG (Table 3). Dexamphetamine was administered and spleens were removed 10 or 25 min later. Ten minutes after dexamphetamine administration there was still a PEG-induced significant depletion of noradrenaline ($P < 0.01$) in the P_2 fraction but the noradrenaline content of the S fraction had returned to control levels. The noradrenaline content of the P_2 fraction had returned to control levels after a further 15 minutes. The repletion of noradrenaline in the S fraction thus coincided with the restoration of noradrenaline overflow in response to splenic nerve stimulation.

PEG (5 mg/kg) treatment in dexamphetamine-pretreated animals

Plasma noradrenaline. The administration of dexamphetamine (2.5 mg/kg) 40 min before the injection of PEG (5 mg/kg) prevented the latter from abolishing the overflow of noradrenaline in response to splenic nerve stimulation (Figure 2a).

The rise in blood pressure invariably observed after administration of PEG was absent in animals previously treated with dexamphetamine. In these animals no elevation of the noradrenaline concentration of resting samples was produced by PEG.

Splenic noradrenaline. Pretreatment of cats for 40 min with dexamphetamine (2.5 mg/kg) prevented the PEG-induced noradrenaline depletion from the P_2 and S fractions (Table 3).

PEG treatment (15 mg/kg)

Plasma noradrenaline. Treatment with this dose of PEG abolished the overflow of noradrenaline in response to splenic nerve stimulation at 15 and 30 min after its administration (Figure 2d). A similar result was obtained in two experiments in cats which had been treated 18 h previously with PEG.

Fifteen minutes after the injection of PEG there was a significant increase ($P < 0.001$) in the resting plasma noradrenaline concentration of splenic venous blood. The result (mean ± s.e. mean, $n = 3$) was: 10 ± 0.6 ng/ml.

Splenic noradrenaline

PEG produced a significant depletion ($P < 0.05$) of noradrenaline in only the S fraction of spleens at 30 min and at 18 h after its administration (Table 4). These results contrast with the effects of PEG (5 mg/kg) where depletions of noradrenaline were found in both P_2 and S fractions at these times.

Dexamphetamine treatment in PEG (15 mg/kg)-pretreated animals

Plasma noradrenaline. Administration of dexamphetamine (2.5 mg/kg) to cats pretreated for 30 min with PEG (15 mg/kg) produced a restoration of noradrenaline overflow in response to nerve stimulation 25 and 40 min after dexamphetamine treatment (Figure 2d). These results contrast with those obtained in similar experiments with the lower dose (5 mg/kg) of PEG where a restoration of overflow was obtained as early as 10 min after dexamphetamine treatment.

Splenic noradrenaline. Ten minutes after the administration of dexamphetamine (2.5 mg/kg) to animals pretreated for 30 min with PEG (15 mg/kg) there was a further decrease ($P < 0.05$)

Table 3 Interaction between (−)-*N*-(1-phenylethyl)guanidine (PEG) (5 mg/kg) and dexamphetamine (DAS) (2.5 mg/kg) on the content and subcellular distribution of noradrenaline in cat spleen

	Spleen fraction			
	P_1	P_2	S	T
Treatment	*Noradrenaline content (ng/μmol DNA-P)*			
Saline ($n = 12$)	43 ± 5.1	19 ± 1.3	19 ± 1.5	93 ± 8.7
DAS, 40 min/PEG, 30 min ($n = 4$)	37 ± 4.1	20 ± 2.1	21 ± 2.2	90 ± 4.0
PEG, 30 min/DAS, 10 min ($n = 5$)	35 ± 2.4	17 ± 0.7	20 ± 2.3	101 ± 12.1
PEG, 30 min/DAS, 40 min ($n = 5$)	42 ± 9.0	20 ± 5.1	27 ± 4.9	107 ± 22.0
PEG, 18 h/DAS, 10 min ($n = 5$)	35 ± 6.0	12 ± 1.8**	18 ± 3.9	73 ± 10.8
PEG, 18 h/DAS, 25 min ($n = 5$)	33 ± 5.2	18 ± 1.1	19 ± 3.0	80 ± 11.2

Results quoted are means ± s.e. mean. *n*, number of observations.
** $P < 0.01$.

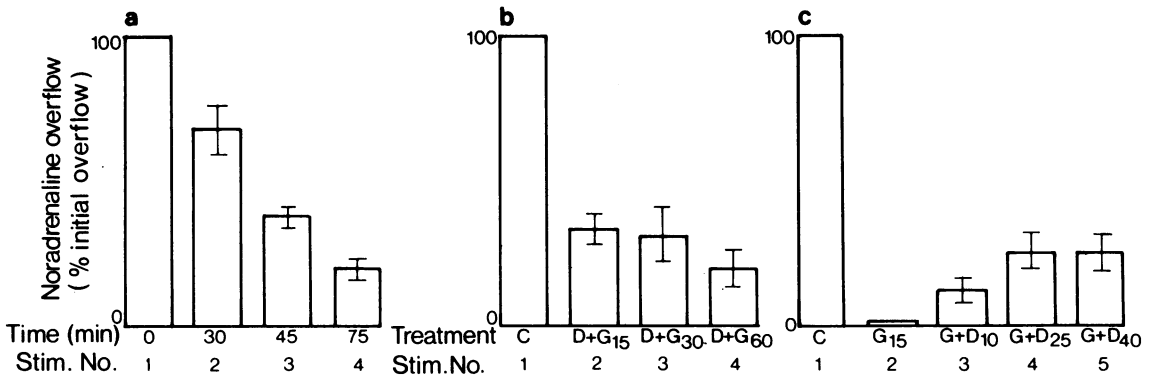

Fig. 3 Effects of guanethidine and its interaction with dexamphetamine on the overflow of noradrenaline during splenic nerve stimulation. Results are means with s.e. mean. (a) Effect of guanethidine (5 mg/kg) administered 7 days before the first stimulation. Time is min after collection of initial stimulation sample. (b) Effect of injection of guanethidine (5 mg/kg) (G) into cats injected 40 min previously with dexamphetamine (2.5 mg/kg) (D); subscripts show time (min) after injection of guanethidine. (c) Effect of injection of dexamphetamine (2.5 mg/kg) into cats injected 15 min previously with guanethidine (5 mg/kg); subscripts following G indicate time (min) after injection of guanethidine; subscripts following G + D indicate time (min) after injection of dexamphetamine into the guanethidine-pretreated cats. C, controls. Numbers of observations: in (a) = 3, in (b) = 5, in (c) = 6.

in the noradrenaline content of the S fraction over and above that produced by PEG alone (Table 4). This lack of refilling of the S fraction with noradrenaline 10 min after dexamphetamine correlates with the lack of restoration of noradrenaline overflow at this time (Figure 2d). Twenty five and 40 min after dexamphetamine, the noradrenaline content of the S fraction had returned to control values (Table 4); this was associated with the restoration of noradrenaline overflow at these times (Figure 2d).

Guanethidine treatment (5 mg/kg)

Plasma noradrenaline. Guanethidine abolished the overflow of noradrenaline in response to nerve stimulation at 15 min, 30 min, 1 h, 18 h, 42 h and 72 h after its administration; the overflow had returned to within the control range after 7 days (Figure 3a).

Guanethidine also significantly increased ($P < 0.01$) the noradrenaline concentration in resting plasma samples at times up to 1 h after its administration. The results (ng/ml) were (means ± s.e., $n = 3$) at 15 min, 30 min and 60 min: 11 ± 1.0; 9 ± 0.6; 5 ± 1.3, respectively. These results contrast with those obtained after PEG where there was a significant elevation in plasma noradrenaline only at 15 min after its administration.

Table 4 The effects of (−)-*N*-(1-phenylethyl)guanidine (PEG) (15 mg/kg) and of the interaction between dexamphetamine (DAS) (2.5 mg/kg) and PEG (15 mg/kg) on the content and subcellular distribution of noradrenaline in cat spleen

	Spleen fraction			
	P_1	P_2	S	T
Treatment	*Noradrenaline content (ng/μmol DNA-P)*			
Saline (n = 12)	43 ± 5.1	19 ± 1.3	19 ± 1.5	93 ± 8.7
PEG, 30 min (n = 6)	48 ± 7.7	20 ± 2.2	13 ± 1.5*	91 ± 8.2
PEG, 18 h (n = 4)	44 ± 4.5	21 ± 1.7	12 ± 1.1*	103 ± 8.3
PEG, 30 min/DAS, 10 min (n = 5)	39 ± 4.0	18 ± 1.0	8 ± 1.0†	76 ± 6.0
PEG, 30 min/DAS, 25 min (n = 4)	48 ± 5.5	20 ± 0.8	19 ± 1.8	98 ± 5.7
PEG, 30 min/DAS, 40 min (n = 4)	41 ± 4.0	20 ± 1.7	19 ± 1.3	85 ± 8.8

Results quoted are means ± s.e. mean. n, number of observations.
* $P < 0.05$; † $P < 0.001$.

Splenic noradrenaline. Guanethidine produced a significant depletion of noradrenaline from the P_2 and S fractions at all times studied between 15 min and 72 h (Table 5). The noradrenaline content of these two fractions had returned to within the control range after 7 days (Table 5). Unlike PEG and bretylium (Abbs & Robertson, 1970), however, guanethidine caused a progressive decline in the noradrenaline content of all the subcellular fractions; this effect was maximal after 18 h (Table 5).

Guanethidine (5 mg/kg) treatment in dexamphetamine-pretreated animals

Plasma noradrenaline. Pretreatment of cats for 40 min with dexamphetamine (2.5 mg/kg) prevented guanethidine from abolishing noradrenaline overflow (Figure 3b). However, the noradrenaline overflowing in response to stimulation 2 was reduced when compared with analogous overflow values in cats which had received saline (Fig. 1a) or dexamphetamine alone (Figure 1b).

The pressor response and the elevation of plasma noradrenaline levels which invariably followed the injection of guanethidine were not observed in dexamphetamine-pretreated animals.

Splenic noradrenaline. Pretreatment for 40 min with dexamphetamine (2.5 mg/kg) prevented guanethidine from causing a loss of noradrenaline from subcellular fractions of cat spleen (Table 6).

Dexamphetamine treatment in guanethidine (5 mg/kg)-pretreated animals

Plasma noradrenaline. Dexamphetamine (2.5 mg/kg) was injected into cats 15 min after the administration of guanethidine (5 mg/kg). Dexamphetamine increased the overflow of

Table 5 The effect of guanethidine (5 mg/kg) on the content and subcellular distribution of noradrenaline in cat spleen

	Spleen fraction			
	P_1	P_2	S	T
Treatment	Noradrenaline content (ng/μmol DNA-P)			
Saline (n = 12)	43 ± 5.1	19 ± 1.3	19 ± 1.5	93 ± 8.7
Guanethidine, 15 min (n = 7)	42 ± 6.4	13 ± 2.2*	11 ± 3.7*	76 ± 11.4
Guanethidine, 30 min (n = 6)	34 ± 2.5	12 ± 1.6**	11 ± 1.5**	64 ± 2.7*
Guanethidine, 60 min (n = 5)	35 ± 2.0	8 ± 0.6†	7 ± 0.7†	64 ± 2.5*
Guanethidine, 18 h (n = 4)	12 ± 4.5**	undetectable	undetectable	16 ± 5.9†
Guanethidine, 48 h (n = 3)	16 ± 1.9*	5 ± 1.0†	5 ± 1.4†	31 ± 5.4†
Guanethidine, 72 h (n = 3)	31 ± 9.4	8 ± 1.6**	10 ± 0.7**	57 ± 5.1*
Guanethidine, 7 days (n = 4)	50 ± 5.7	19 ± 3.4	18 ± 1.9	112 ± 13.1

Results quoted are means ± s.e. mean. n, number of observations.
* $P < 0.05$; ** $P < 0.01$; † $P < 0.001$.

Table 6 Interaction between guanethidine (5 mg/kg) and dexamphetamine (DAS) (2.5 mg/kg) on the content and subcellular distribution of noradrenaline in cat spleen

	Spleen fraction			
	P_1	P_2	S	T
Treatment	Noradrenaline content (ng/μmol DNA-P)			
Saline (n = 12)	43 ± 5.1	19 ± 1.3	19 ± 1.5	93 ± 8.7
DAS, 40 min/ guanethidine, 60 min (n = 5)	37 ± 3.2	20 ± 0.8	18 ± 1.9	78 ± 10.9
Guanethidine, 15 min/DAS, 10 min (n = 6)	31 ± 4.4	11 ± 1.4**	16 ± 2.1	59 ± 8.4*
Guanethidine, 15 min/DAS, 25 min (n = 4)	36 ± 2.5	18 ± 1.8	19 ± 0.6	78 ± 5.1
Guanethidine, 15 min/DAS, 40 min (n = 4)	45 ± 5.2	22 ± 1.4	21 ± 1.2	91 ± 9.2

Results quoted are means ± s.e. mean. n, number of observations.
* $P < 0.05$; ** $P < 0.01$.

noradrenaline to about 12% of the initial level after 10 min; the overflow was further restored, to approximately 30% of the initial level, at 25 and 40 min after dexamphetamine (Figure 3c).

When animals were treated for longer periods (1 h and 18 h) with guanethidine, dexamphetamine treatment failed to restore noradrenaline overflow.

Splenic noradrenaline. Ten minutes after the administration of dexamphetamine (2.5 mg/kg) to cats injected 15 min previously with guanethidine (5 mg/kg) there was a restoration of the noradrenaline content of the S fraction to control values (Table 6). This was not accompanied by a restoration of the noradrenaline content of the P_2 fraction; this restoration took longer and was complete at 25 min after dexamphetamine administration.

Thus the recovery of noradrenaline overflow after dexamphetamine treatment is associated with a restoration of the noradrenaline content of the S fraction; the P_2 fraction refills later.

Guanethidine treatment (15 mg/kg)

Plasma noradrenaline. The overflow of noradrenaline in response to nerve stimulation was abolished 15, 30 and 60 min after the administration of guanethidine.

There was an elevation of the resting noradrenaline concentration of plasma from splenic venous blood 15, 30 and 60 min after this dose of the drug. The results, expressed as ng/ml (means ± s.e. mean, $n = 3$) were: 13 ± 1.2; 9 ± 1.9; 6 ± 2.2, respectively. The magnitude of this effect was similar to that produced by 5 mg/kg of the drug.

Splenic noradrenaline. Thirty minutes after guanethidine there was a significant depletion of noradrenaline from only the S fraction of cat spleen (Table 7). This result contrasts with those found after 5 mg/kg of the drug when there was a depletion of noradrenaline from both the P_2 and S fractions at this time.

Debrisoquine treatment

Plasma noradrenaline. The overflow of noradrenaline was not abolished 15 min after the administration of debrisoquine (5 mg/kg); overflow was approximately 30% of the initial overflow.

However, transmitter overflow had declined to undetectable levels 30 min and 1 h after debrisoquine treatment. There was a significant increase in the noradrenaline concentration of resting plasma samples 15 min after the injection of debrisoquine. The result was (mean ± s.e. mean, $n = 3$): 11 ± 2.6 ng/ml.

Splenic noradrenaline. There was a significant decrease in the noradrenaline content of both the P_2 and S fractions 30 min after the injection of debrisoquine (5 mg/kg). There were no significant differences in the mean noradrenaline contents of the P_1 and T fractions when compared with saline-injected animals (Table 7). These results contrast with the results of Robertson & Abbs (1971) who found that, with a large dose (10 mg/kg) of debrisoquine, there was a selective depletion of noradrenaline from only the supernatant fraction after 30 min; there was no overflow of noradrenaline at this time.

Discussion

The intravenous injection of either PEG, guanethidine or debrisoquine produced sympathomimetic effects, such as contraction of the splenic capsule, a rise in arterial blood pressure, and an increase in the noradrenaline concentration of splenic venous blood; this latter increase was of

Table 7 Content and subcellular distribution of noradrenaline in cat spleen 30 min after the administration of guanethidine (15 mg/kg) or debrisoquine (5 mg/kg)

	Spleen fraction			
	P_1	P_2	S	T
Treatment	*Noradrenaline content (ng/μmol DNA-P)*			
Saline ($n = 12$)	43 ± 5.1	19 ± 1.3	19 ± 1.5	93 ± 8.7
Guanethidine ($n = 6$)	47 ± 6.7	20 ± 3.0	12 ± 2.4*	85 ± 16.2
Debrisoquine ($n = 4$)	47 ± 4.9	12 ± 1.1**	11 ± 0.9**	90 ± 7.3

Results quoted are means ± s.e. mean. n, number of observations.
* $P < 0.05$; ** $P < 0.01$.

similar magnitude after all the compounds and was not dose-related. These actions are probably due to the rapid uptake of the adrenergic neurone-blocking agents into adrenergic nerve terminals (Boura, Copp, Duncombe, Green & McCoubrey, 1960; Bisson & Muscholl, 1962; Schanker & Morrison, 1965; Giachetti & Shore, 1967) and the displacement of stored noradrenaline which is released into the bloodstream. Shortly (15-30 min) after the administration of 5 mg/kg of each of these compounds there was a significant depletion of noradrenaline from both P_2 and S fractions of cat spleen; this loss of noradrenaline was temporally correlated with the adrenergic neurone-blocking action of the compounds.

However, the release of noradrenaline *per se* is unlikely to account for the subcellular depletion of noradrenaline because dexamphetamine, which releases similar amounts of noradrenaline into splenic venous blood, does not produce subcellular depletion of the transmitter, neither does it produce adrenergic neurone blockade. There must therefore be an additional mechanism involved, such as interference with the replacement of the displaced transmitter.

The adrenergic nerves were blocked with 5 mg/kg of the compounds only when there was a depletion of noradrenaline from the P_2 and S fractions. When the noradrenaline content had returned to control levels, 7 days after treatment, nerve function was also restored. When the subcellular depletion of noradrenaline was prevented by pretreatment with dexamphetamine, adrenergic neurone blockade did not occur. These results are similar to those reported by Abbs & Robertson (1970) for bretylium, except that in the present experiments it was not possible to demonstrate a selective loss of noradrenaline from the S fraction before a similar loss occured in the P_2 fraction. Experiments in which the adrenergic neurone blockade, produced by the guanidines, was antagonized by dexamphetamine did however provide evidence that it is the integrity of the noradrenaline content of the S fraction that is essential for the proper functioning of adrenergic nerves. For example, when the adrenergic neurone blockade produced by 5 mg/kg PEG or guanethidine was antagonized, after approximately 18 h or after 15 min respectively, by dexamphetamine, the restoration of nerve function was accompanied by a selective repletion of the noradrenaline content of the S fraction; only at later times after dexamphetamine treatment was there a restoration of the noradrenaline content of the P_2 fraction. Further support for this contention comes from acute experiments with larger doses (15 mg/kg) of PEG or guanethidine or debrisoquine (10 mg/kg) (Robertson & Abbs, 1971). In these experiments there was a selective depletion of noradrenaline from the S fraction and adrenergic nerve function was impaired; the noradrenaline content of the P_2 fraction remained within the control range.

The magnitude of depletion of noradrenaline from the S fraction was similar after both high and low doses of PEG, guanethidine and debrisoquine; this loss of noradrenaline represents approximately 8% of the total tissue content of transmitter.

It has been suggested (Campos, Stitzel & Shideman, 1963; Abbs & Robertson, 1970) that an equilibrium may exist *in vivo* between the 'stores' of noradrenaline which give rise to the supernatant and microsomal (particulate) fractions of homogenates from adrenergically-innervated tissues. A transference of noradrenaline from the microsomal fraction to the supernatant fraction may therefore occur if this equilibrium were disturbed by, for example, the establishment of a concentration gradient. Low doses (5 mg/kg) of PEG, guanethidine and debrisoquine may release noradrenaline from the supernatant fraction and thus establish such a concentration gradient. Noradrenaline would then be transferred from the P_2 fraction into the S fraction in an attempt to restore the equilibrium but, if the compounds prevented the S fraction from retaining the transmitter, this amine too may leak out of the neurone. Adrenergic neurone blockade may thus occur because transmitter has been lost from the supernatant fraction and under these circumstances would be accompanied by a loss of noradrenaline from the P_2 fraction.

A similar situation may occur with bretylium but in the experiments of Abbs & Robertson (1970) the equilibrium disturbance may have had a different time course because they were able to demonstrate a greater (significant) depletion in the supernatant fraction than in the P_2 fraction at the onset of blockade.

When the S fraction refills with noradrenaline after antagonism of the adrenergic neurone blockade by dexamphetamine the equilibrium is no longer disturbed; this allows the P_2 fraction to refill with noradrenaline.

The larger doses of the guanidines, 15 mg/kg PEG and guanethidine, and 10 mg/kg debrisoquine (Robertson & Abbs, 1971) may, in addition to producing loss of noradrenaline from the S fraction and adrenergic neurone blockade, have a stabilizing effect on the noradrenaline 'store' which gives rise to the P_2 fraction on homogenization because they produce adrenergic neurone blockade without a concomitant loss of noradrenaline from the P_2 fraction. This stabilizing action would prevent the transference

of noradrenaline from the P_2 fraction to the S fraction in response to the establishment of a concentration gradient.

In contrast to PEG and bretylium (Abbs & Robertson, 1970), guanethidine produces a further substantial depletion of noradrenaline which becomes maximal approximately 18 h after the establishment of its adrenergic neurone-blocking action. This further loss of transmitter may be mainly in the form of deaminated metabolites (Harrison, Chidsey, Goldman & Braunwald, 1963) which may indicate that guanethidine causes transmitter loss from different sites or by different mechanisms, the site or mechanism depending on the duration of treatment.

It was originally suggested (Shepherd & Zimmermann, 1959; Cass *et al.*, 1960; Burn, 1961) that the ability of guanethidine to produce adrenergic neurone blockade may be connected with its ability to produce a substantial loss of transmitter from adrenergically-innervated tissues. The present results do not support this suggestion but agree with the observations of Cass & Spriggs (1961), Gaffney *et al.* (1963) and Spriggs (1966) who found that adrenergic neurone blockade was demonstrable before any significant reduction in the total tissue content of noradrenaline was observed.

When the noradrenaline 'stores' are partitioned by subcellular fractionation it becomes apparent that guanethidine, like PEG, debrisoquine and bretylium (Abbs & Robertson, 1970), initially produces a subcellular depletion of noradrenaline which is temporally correlated with its adrenergic neurone-blocking action. The further depletion of noradrenaline produced by this drug is unlikely to be the cause of its adrenergic neurone-blocking activity although it may contribute to its maintenance.

We are grateful to Dr R. Fielden of the Smith, Kline & French Research Institue, Welwyn Garden City for the generous supply of (−)-*N*-(1-phenylethyl) guanidine sulphate, and to Roche Products Ltd for the gift of debrisoquine.

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(Received September 9, 1973)