Br. J. Pharmac. (1976), **58**, 497–504

EXTRA-VESICULAR BINDING OF NORADRENALINE AND GUANETHIDINE IN THE ADRENERGIC NEURONES OF THE RAT HEART: A PROPOSED SITE OF ACTION OF ADRENERGIC NEURONE BLOCKING AGENTS

A. GIACHETTI & R.A. HOLLENBECK[1]

Department of Pharmacology, University of Texas Southwestern Medical School, Dallas, Texas, U.S.A.

1 The binding and efflux characteristics of [^{14}C]-guanethidine and [^{3}H]-noradrenaline were studied in heart slices from rats which were pretreated with reserpine and nialamide.

2 Binding of both compounds occurred at extra-vesicular sites within the adrenergic neurone. After a brief period of rapid washout, the efflux of [^{14}C]-guanethidine and [^{3}H]-noradrenaline proceeded at a steady rate. The efflux of both compounds appeared to occur from a single intraneuronal compartment.

3 (+)-Amphetamine accelerated the efflux of [^{14}C]-guanethidine and [^{3}H]-noradrenaline; this effect was inhibited by desipramine.

4 Unlabelled guanethidine and amantadine also increased the efflux of labelled compounds. Cocaine in high concentrations increased slightly the efflux of [^{14}C]-guanethidine but not that of [^{3}H]-noradrenaline.

5 Heart slices labelled with [^{3}H]-noradrenaline became refractory to successive exposures to releasing agents although an appreciable amount of labelled compound was still present in the slices.

6 It is suggested that [^{14}C]-guanethidine and [^{3}H]-noradrenaline are bound at a common extra-vesicular site within the adrenergic neurone. Binding of guanethidine to the extra-vesicular site may be relevant to its pharmacological action, i.e., the blockade of adrenergic transmission.

Introduction

In an attempt to elucidate the mechanism of action of adrenergic neurone blockers Abbs and colleagues (Abbs & Robertson, 1970; Abbs & Pycock, 1973; Abbs & Dodd, 1974) studied the intracellular distribution of noradrenaline in the spleen and in heart following the administration of bretylium and certain guanidinium compounds to cats and rats. These studies revealed significant changes in the intra-neuronal distribution of transmitter, which were temporally related to the onset of adrenergic neurone blockade; however, it is difficult to assign a functional role to subcellular fractions of tissue homogenates since they may not correspond to functional sites existing in intact tissues.

An insight into the intraneuronal locus of action of adrenergic neurone blockers may be obtained by examining the interactions of these agents with amphetamine, a drug which readily prevents and reverses the blockade of adrenergic transmission (Laurence & Rosenheim, 1960; Day & Rand, 1963).

Since amphetamine displaces noradrenaline mainly from an extra-vesicular compartment (Farnebo, 1971) it is conceivable that the ability to prevent or reverse the adrenergic blockade and the noradrenaline releasing properties are causally related and effected at the same site.

Other observations suggest that adrenergic neurone-blocking agents may induce a blockade of impulse transmission through an action on an extra-vesicular site within the neurone. For example, Shand, Morgan & Oates (1973), by examining the nerve-induced release of guanethidine and bethanidine from adrenergic nerve endings, indicated that the portion of guanidinium compounds retained in storage vesicles does not contribute to the development of adrenergic blockade and they postulated an extra-vesicular site of action for adrenergic neurone-blocking drugs.

In view of the evidence implicating a possible interaction of adrenergic neurone blockers with extra-vesicular stores of noradrenaline in the production of

[1] Present address: Lab. of Preclinical Pharmacology, N.I.M.H., St. Elizabeth's Hospital, Washington, D.C. 20032, U.S.A.

adrenergic blockade, we have examined the characteristics of guanethidine and noradrenaline binding at extra-vesicular sites within the adrenergic neurone and factors which influence that binding.

Methods

Preparation and incubation of rat heart slices

Female Sprague-Dawley rats (150–200 g) were used. Reserpine (2 mg/kg) and nialamide (100 mg/kg), when given, were administered subcutaneously 18 and 3 h, respectively, before killing. Heart ventricle slices (0.3 mm, 50–80 mg) were obtained with a Harvard tissue slicer. The slices were incubated in Krebs-Ringer bicarbonate (pH 7.4) supplemented with tricarboxylic acid intermediates (Dengler, Spiegel & Titus, 1961) as well as ascorbic acid (50 mg/litre). After equilibration (15 min) slices were incubated with labelled noradrenaline ((±)-[^{3}H]-noradrenaline) or guanethidine ([^{14}C]-guanethidine). Due to the difference in specific activity between these compounds (see below), and in order to obtain tissue levels of isotope adequate to perform efflux studies, heart slices were incubated with 0.1 μM [^{3}H]-noradrenaline for 30 min or with 1 μM [^{14}C]-guanethidine for 60 minutes. All incubations and washouts were performed at 37° in the presence of 95% O_2 and 5% CO_2.

Efflux of labelled compounds

After incubation, the efflux of labelled compounds from the heart slices was followed by placing the tissue in 5 ml of the buffer and transferring to fresh buffer at 5 min intervals for periods of up to 70 minutes.

The ability of drugs to release labelled compounds from the slices was studied by including the test drug in the washout medium for brief periods of time. Thus, slices labelled with [^{3}H]-noradrenaline were exposed to drugs for a single 5 min interval after 55 min of washout, unless otherwise indicated. In experiments involving the release of [^{14}C]-guanethidine, slices were exposed to drugs after 30 min of efflux and the exposure time was prolonged to 3 periods of 5 min each. This longer exposure was necessary in order to obtain levels of radioactive compound in the medium significantly higher than the baseline efflux. It should be mentioned that baseline efflux was subtracted from each sample (see calculations).

The effect of a membrane transport inhibitor on release of labelled compounds was investigated by adding desipramine (10 μM) to the washout medium for 10 min before and during the interval of exposure to the releasing agent.

Analytical determinations

Radioactivity in the washout medium was determined by the addition of 2 ml of medium to 10 ml scintillation fluid (Aquasol, New England Nuclear) and counting in a liquid scintillation spectrometer (Beckman) to a statistical accuracy of ±3%. The efficiency of counting averaged 39% for ^{3}H and 64% for ^{14}C.

Tissue levels of labelled noradrenaline were determined by homogenization of the tissue in 29 parts of 0.4 N $HClO_4$ followed by centrifugation. Aliquots (0.5 ml) of the supernatant were placed in 10 ml scintillation solution and counted as above. In preliminary experiments incubation medium and perchloric acid extracts were absorbed onto alumina and eluted as described by Anton & Sayre (1962). The amount of radioactivity in the eluate after correction for recovery (53.4%) was equal to the radioactivity present before purification, thus indicating that total radioactivity represented unchanged [^{3}H]-noradrenaline. Results are therefore based on total radioactivity. Oxidative deamination can be excluded *a priori* in the present experiments since a monoamine-oxidase inhibitor was always employed.

[^{14}C]-guanethidine sulphate was purified before use by extraction into butanol-heptane mixture as described by Medina, Giachetti & Shore (1969) for debrisoquine and purity was confirmed by ascending paper chromatography.

In experiments involving labelled guanethidine, radioactivity was determined directly in the incubation medium; tissue levels were determined by the same procedure described for [^{3}H]-noradrenaline.

Calculations

The uptake of labelled compounds is expressed in terms of the ratio of radioactivity in the tissue (ct min^{-1} g^{-1}) corrected for cell water (85%) to that in the incubation medium (ct min^{-1} ml^{-1}). Rate of efflux of labelled compounds during washout is expressed as percentage efflux with time and was calculated as follows:

$$\% \text{ efflux} = \frac{\text{ct/min released during 5 min sample interval}}{\text{ct/min present in tissue at beginning of sample interval}} \times 100$$

To determine the level of labelled compound remaining in the tissues at any given 5 min sampling interval during washout, the total ct/min determined for each 5 min sample was added successively to the final tissue ct/min level, beginning with the last washout sample and ending with the first. Percentage efflux is a rate function and represents that portion of labelled compound released during a 5 min washout

interval relative to the total radioactivity present in the tissue slice at the beginning of that interval.

In the case of tissue slices which were subjected to the amine-releasing activity of drugs, percentage release is expressed as the difference in percentage efflux between the 5 min drug exposure interval and the preceding 5 min washout interval. The latter value represents a point at which the washout of labelled agent proceeds at a constant rate (baseline).

Drugs

Compounds used in the investigation included (+)-amphetamine sulphate, amantadine hydrochloride, guanethidine sulphate, cocaine hydrochloride, desipramine hydrochloride (DMI), nialamide base, reserpine phosphate, potassium chloride; (±)-[7-^{3}H]-noradrenaline, 13 Ci/mM (New England Nuclear) and [^{14}C]-guanethidine sulphate, 4.1 mCi/mM (Ciba-Geigy).

Results

Accumulation of [^{14}C]-guanethidine and [^{3}H]-noradrenaline by rat ventricular slices

Incubation of heart slices with [^{14}C]-guanethidine or [^{3}H]-noradrenaline resulted in the rapid accumulation of both compounds within the slices as indicated by the ratios of tissue concentration relative to the medium concentration of compounds (Table 1).

Reserpine pretreatment decreased very considerably the accumulation of noradrenaline even when tissue monoamine-oxidase was inhibited by nialamide. In contrast, the initial uptake of [^{14}C]-guanethidine was not affected although the overall accumulation noted at longer incubation times was reduced (Table 1).

To ensure that the labelled compounds were accumulated within the adrenergic neurone when vesicular storage was impaired by reserpine, cocaine (10 μM), a potent inhibitor of the amine pump, was included in the incubation medium. In these conditions the accumulation of the [^{3}H]-noradrenaline was inhibited by 90%, while that of [^{14}C]-guanethidine was inhibited by 51%; this different sensitivity to the action of cocaine suggests that part of the [^{14}C]-guanethidine accumulates in non-neuronal cells.

These results indicate that in the absence of vesicular storage function, the adrenergic neurone maintains the ability to accumulate these compounds in the axoplasm where binding occurs at extra-vesicular sites.

The following series of experiments illustrates the latter point.

Efflux of [^{14}C]-guanethidine and [^{3}H]-noradrenaline

Heart slices taken from reserpine-treated rats were allowed to accumulate [^{14}C]-guanethidine or [^{3}H]-noradrenaline and then transferred at regular intervals of time to tracer-free medium. The characteristics of the efflux of [^{14}C]-guanethidine and [^{3}H]-noradrenaline from heart slices are depicted in Figure 1a. In this graph the radioactivity appearing in the medium is expressed as percentage of efflux against time (see Methods section), i.e. the graph represents the fractional rate of efflux of isotopic compounds from the slices. Analysis of Figure 1 reveals an initial rapid rate of efflux of each compound lasting 10–15 min followed by a very slow and steady rate which amounts to 7–10% efflux per 5 minutes. This latter phase suggests that efflux of compounds is mainly from a single intracellular compartment.

Another indication that [^{14}C]-guanethidine exists in the slice in two kinetically different compartments is seen in the lower portion of Figure 1 where the isotope remaining in the tissue at the end of 5 min intervals, expressed as percentage of total taken up, is plotted against time. The same desaturation pattern is observed for [^{3}H]-noradrenaline (Figure 1, lower

Table 1 Accumulation of [^{14}C]-guanethidine ([^{14}C]-G) and [^{3}H]-noradrenaline ([^{3}H]-NA) by rat heart slices: effect of reserpine treatment

	Tissue to medium ratios ± s.e. mean			
Incubation time (min)	*Control*		*Reserpine*	
	[^{14}C]-G	*[^{3}H]-NA*	*[^{14}C]-G*	*[^{3}H]-NA*
10	1.15 ± 0.093	1.91 ± 0.091	1.20 ± 0.041	0.66 ± 0.027
30	2.21 ± 0.082	2.17 ± 0.037	1.89 ± 0.122	1.17 ± 0.084
60	3.53 ± 0.367	5.19 ± 0.420	2.68 ± 0.163	1.58 ± 0.099

Each result is the mean of 3–5 experimental values. Reserpine-treated rats received 2 mg/kg subcutaneously, and nialamide (100 mg/kg s.c.), 18 h and 3 h respectively before they were killed. Control designates rats receiving nialamide (100 mg/kg s.c.) 3 h before they were killed. Slices were incubated with 0.1 μM [^{3}H]-noradrenaline or with 1 μM [^{14}C]-guanethidine as described in the Methods section.

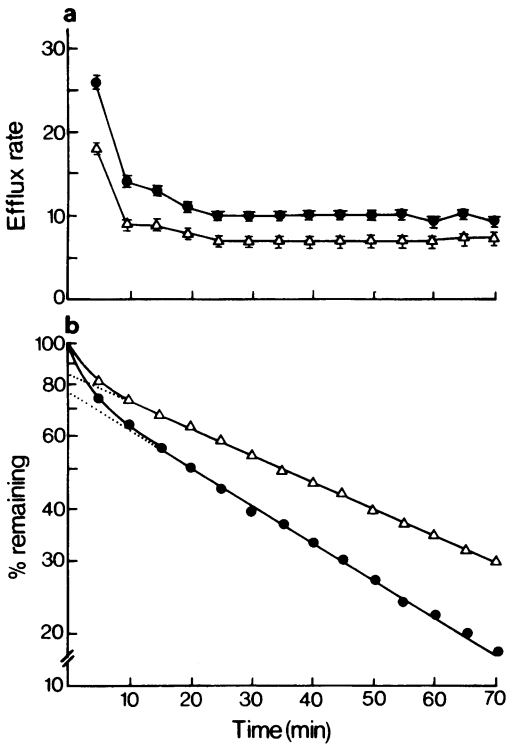

Figure 1 Rate of efflux (a) of [^{3}H]-noradrenaline (●) and [^{14}C]-guanethidine (△) during washout, and (b) semilogarithmic plot of labelled compounds remaining in rat heart slices during washout expressed as percentage of total isotope taken up. Rate is expressed as percentage of isotope present which leaves the tissues during 5 min period (see Methods section). Dotted lines in plot (b) are extrapolations to t=0 to determine half-times of desaturation. Heart slices in this and following experiments were taken, unless otherwise indicated, from rats treated with reserpine (2 mg/kg s.c. 18 h) and nialamide (100 mg/kg s.c. 3 h). Each point is the mean of 6–8 experiments. Vertical lines show s.e. mean.

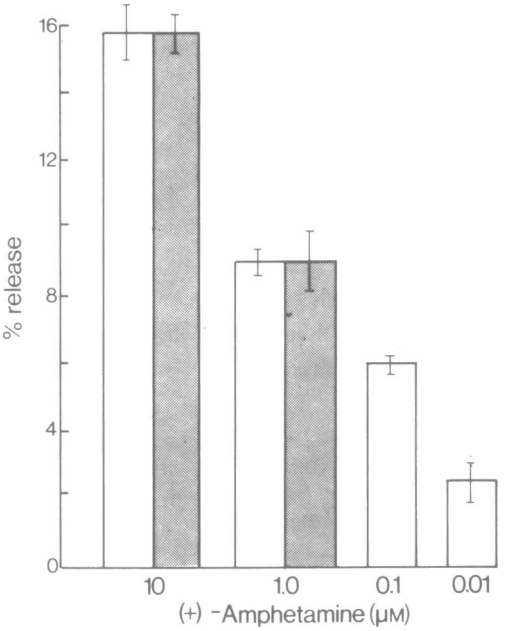

Figure 2 Effect of amphetamine on efflux of [^{3}H]-noradrenaline (open columns) or ([^{14}C]-guanethidine (solid columns) from heart slices. Release expresses the portion of efflux of labelled compound above baseline washout when concentrations of (+)-amphetamine were present for 5 min ([^{3}H]-noradrenaline) or 15 min ([^{14}C]-guanethidine). Each column is the mean of 6–8 observations. Vertical lines show s.e. mean.

panel). Extrapolation of the slow component of each curve allows the estimation of the half-time for efflux of [^{3}H]-noradrenaline (33 min) and [^{14}C]-guanethidine (48 minutes). In the early phase of washout, the efflux is too rapid to permit an accurate determination of half-time of efflux.

Release of [^{14}C]-guanethidine and [^{3}H]-noradrenaline by amphetamine

To examine the ability of amphetamine to induce the release of accumulated [^{14}C]-guanethidine and [^{3}H]-noradrenaline from reserpine-treated rat tissue, heart slices were exposed to amphetamine for 15 or 5 min, respectively, at a point during washout when efflux of either labelled compound had reached a steady rate. The release expressed as percentage efflux of the labelled compounds above baseline efflux is shown in Figure 2. The extent of release induced by amphetamine is similar for [^{14}C]-guanethidine and [^{3}H]-noradrenaline and, in the range of concentrations examined, appears to be related to the molarity of amphetamine.

The overall amount of amphetamine-releasable noradrenaline was quite small (accounting for about 6% of the noradrenaline taken up by the tissue) and the pool apparently was fully depleted during the interval of amphetamine exposure. In fact, a second exposure to amphetamine (10 μM) 25 min after the first exposure failed to promote release of the labelled compound although the slices still contained appreciable amounts of radioactivity. In other experiments we investigated the ability of amphetamine to release [^{14}C]-guanethidine and [^{3}H]-noradrenaline accumulated in heart slices taken from untreated rats. The results shown in Table 2 indicating enhancement of the releasing properties of amphetamine in reserpine-treated slices relative to

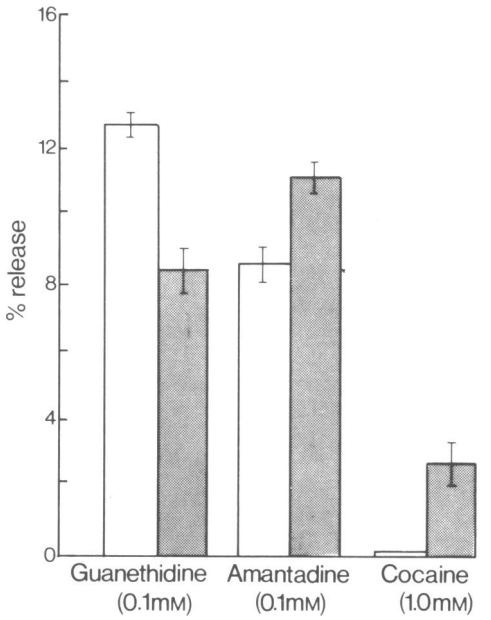

Figure 3 Effect of drugs on efflux of [^{3}H]-noradrenaline (open columns) and [^{14}C]-guanethidine (solid columns). Release expressed as in Figure 2. Drugs were present in washout medium for 5 min ([^{3}H]-noradrenaline) or 15 min ([^{14}C]-guanethidine). On the abscissa scale guanethidine (0.1 mM) represents unlabelled compound. Each column is the mean of 6 observations. Vertical lines show s.e. mean.

Table 2 Release of labelled guanethidine or noradrenaline by amphetamine: effect of reserpine pre-treatment

	% Release above baseline efflux	
Treatment	*[^{14}C]-G*	*[^{3}H]-NA*
Control	4.6 ± 0.17	2.75 ± 0.12
Reserpine*	15.7 ± 0.59	15.7 ± 0.87

Each value is the mean of 4 to 6 experiments ± s.e. mean. Slices were labelled and subsequently exposed to amphetamine (10 μM) as described in the Methods section.
* Reserpine (2 mg/kg s.c.) 18 h before rats were killed. Control and reserpine-treated rats received nialamide (100 mg/kg s.c.) 3 h before killing.

normal slices suggest that reserpine treatment unmasks an amphetamine-releasable pool of either compound.

Release of [^{14}C]-guanethidine and [^{3}H]-noradrenaline by other agents

The ability of unlabelled guanethidine and of the anti-parkinson agent amantadine to release [^{14}C]-guanethidine and [^{3}H]-noradrenaline accumulation in heart slices taken from rats pre-treated with reserpine and nialamide (see Methods section) was also examined. The results summarized in Figure 3 show that both agents promote release of the labelled compounds although they are much weaker than amphetamine.

Since guanethidine and amantadine are known to inhibit the membrane amine transport mechanism (Mitchell & Oates, 1970; Farnebo, Fuxe, Goldstein, Hamberger & Ungerstedt, 1971) it was of interest to determine whether the increase in efflux of labelled compounds could be due to blockade of their reuptake through the neuronal membrane. However, cocaine, a potent amine uptake inhibitor, when tested at 0.1 mM proved to be ineffective as a releaser of [^{3}H]-noradrenaline and promoted release of [^{14}C]-guanethidine only to a minor extent (Figure 3).

To determine whether amphetamine and

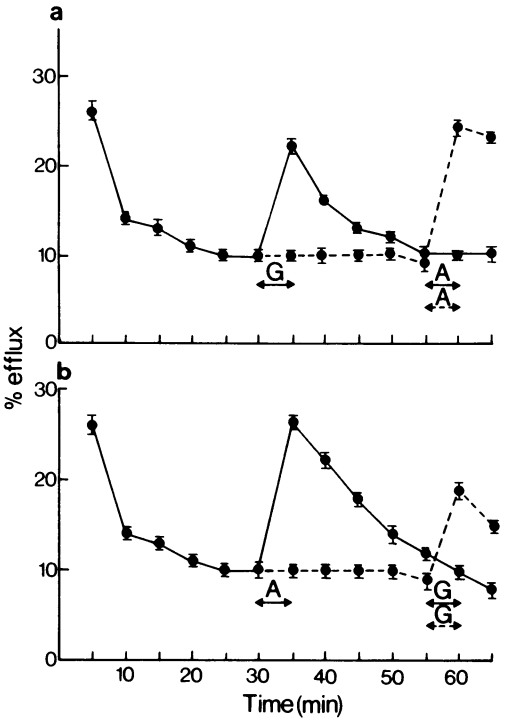

Figure 4 Efflux of [^{3}H]-noradrenaline from rat heart slices: (a) effect of exposure to unlabelled guanethidine (G 0.1 mM) and later to (+)-amphetamine (A 10 μM). Drugs were present at times indicated by solid arrow. In (b) exposure to (+)-amphetamine and later to unlabelled guanethidine. Dashed lines and arrows represent efflux in heart slices exposed to a single releasing agent at 55 min during washout. Percent efflux expressed as in Figure 1 (mean of 4 observations for both (a) and (b); vertical lines show s.e. mean).

guanethidine, when used as releasing agents, were acting upon the same pool of accumulated labelled compound, a series of experiments was performed in which tissue slices loaded with [^{3}H]-noradrenaline were exposed to either amphetamine (10 μM) or guanethidine (0.1 mM) followed by a later exposure to the agents in reverse order. Figure 4 shows that both agents promote labelled amine efflux upon initial exposure, while a later challenge with the alternate agent fails to produce additional release. Thus, guanethidine (0.1 mM) increased the efflux of [^{3}H]-noradrenaline (12% above baseline efflux) whereas exposure to amphetamine (10 μM) in the same preparation after 55 min of wash promoted no additional release of isotope (Figure 4a). The same pattern of release was observed when exposure of the tissues to the releasing agents was reversed (Figure 4b). Failure to promote efflux of [^{3}H]-noradrenaline by a second exposure to a releasing agent was apparently not due to disappearance of labelled compound from the slice, since analysis of these tissues revealed a level of isotope comparable to slices which had been challenged by a single exposure.

Effect of membrane transport inhibition on amphetamine-induced release of [^{14}C]-guanethidine and [^{3}H]-noradrenaline

When heart tissue slices loaded with [^{14}C]-guanethidine or [^{3}H]-noradrenaline were exposed to desipramine (10 μM) for 10 min before and during exposure to amphetamine (10 μM), during washout, inhibition of release was observed (Table 3). Since desipramine was incapable of promoting release in these conditions, it appears that inhibition of release might be due to inability of amphetamine to gain access to the releasable store of labelled compounds.

Table 3 Release of [^{14}C]-guanethidine ([^{14}C]-G) and [^{3}H]-noradrenaline ([^{3}H]-NA) by (+)-amphetamine from rat heart slices: effect of desipramine

Drug (conc 10 μM)	*% Release above baseline efflux*	
	[^{14}C]-G	*[^{3}H]-NA*
Amphetamine	15.7 ± 0.59	15.7 ± 0.87
Desipramine + amphetamine	11.1 ± 0.62	8.5 ± 0.32
Desipramine	0	0

Results are the mean ± s.e. mean of 5 observations. Heart slices incubated with desipramine for 10 min before challenge with (+)-amphetamine during washout of labelled compounds (see Methods section). Rats treated with reserpine and nialamide as in legend of Table 1.

Discussion

Adrenergic neuronal blocking agents inhibit sympathetic neurotransmission by preventing the release of noradrenaline in response to nerve stimulation (Boura & Green, 1957; Abercrombie & Davies, 1963). It has been suggested that the adrenergic neuronal blockers displace noradrenaline from an intraneuronal pool critical for the liberation of transmitter by nerve impulses (Abbs & Robertson, 1971; Shand *et al.*, 1973; Abbs & Dodd, 1974).

To examine the possibility of an extragranular location as a site for interaction between guanethidine and noradrenaline, the function of the amine storage granules in the adrenergic neurone was inhibited by reserpine administration. Table 1 shows that such treatment reduces the ability of the heart slice to accumulate [^{3}H]-noradrenaline and, to a lesser extent, [^{14}C]-guanethidine; similar findings for the accumulation of guanethidine have been reported previously (Brodie, Chang & Costa, 1965).

Evidence that part of the accumulated [^{14}C]-guanethidine and [^{3}H]-noradrenaline may be bound to a single subcellular site was found in the efflux experiments. A large fraction (about 55%) of the total accumulated labelled compound left the tissues upon washout at a steady rate following the rapid disappearance of unspecifically bound amine. Of that labelled compound remaining in the tissues following the rapid phase of efflux, approximately 7% of the [^{14}C]-guanethidine and 10% of the [^{3}H]-noradrenaline left the tissues every 5 min possibly indicating dissociation from a binding site followed by efflux.

Since amphetamine has been shown to be capable of releasing extragranularly bound noradrenaline (Lundborg & Waldeck, 1971; Farnebo, 1971) as well as reversing adrenergic nerve blockade (Laurence & Rosenheim, 1960; Follenfant & Robson, 1970), the agent was selected as a pharmacological tool to aid in relating noradrenaline binding and release characteristics to those of the adrenergic neuronal blocker, guanethidine. Amphetamine (10 μM) was found capable of releasing about 16% of the total [^{14}C]-guanethidine or [^{3}H]-noradrenaline remaining in the tissue after washout of unspecifically bound amine (Figure 2).

The size of the amphetamine-releasable store of [^{14}C]-guanethidine and [^{3}H]-noradrenaline in the adrenergic neurone is apparently increased by reserpine treatment (Table 2). This enhancement of an amphetamine-releasable amine store has been observed for noradrenaline by other investigators (Farnebo, 1971; Enna & Shore, 1974) and probably reflects a different intraneuronal distribution of labelled compounds accumulated when the granular storage function is inhibited by reserpine.

Further evidence for a common or related intraneuronal binding site for guanethidine and

noradrenaline is found in the experiments testing the ability of unlabelled guanethidine and amantadine (an anti-parkinson agent possessing some sympathomimetic properties) to promote release of accumulated [^{14}C]-guanethidine and [^{3}H]-noradrenaline from heart slices (Figure 3). Amantadine, which can reverse adrenergic neuronal blockade (unpublished observations), also displayed an ability to promote efflux of the labelled compounds, thus strengthening the relevance of the extra-vesicular compartment for the maintenance of the adrenergic neurone blockade. The finding that the [^{3}H]-noradrenaline bound extravesicularly is readily exhausted by a single exposure to amphetamine is indicative of a small and discrete compartment which cannot be refilled by the relatively large pool of isotope contained within the neurone. It is also apparent that unlabelled guanethidine releases [^{3}H]-noradrenaline from the same compartment since its releasing action can be abolished by previous challenge with amphetamine (Figure 4). Failure to elicit further release of [^{3}H]-noradrenaline by a second exposure to amphetamine or guanethidine might be due to binding of the releasing agent to extra-vesicular sites. The results of this study indicate that guanethidine interacts with extra-vesicular sites in a manner similar to that of [^{3}H]-noradrenaline and indeed it might replace the transmitter at these sites. That a similar mechanism might be operant for amphetamine can be inferred by the similarities with the releasing action of guanethidine. It is of interest to note that while desipramine or cocaine do not affect the accumulation of amphetamine in rat tissues (Ross & Renyi, 1964; Thoenen, Huerlimann & Haefely, 1968) desipramine reduces considerably the releasing effect of amphetamine on compounds accumulated extravesicularly (Table 3). These results confirm and extend the work of Lundborg & Waldeck (1971) who suggested that extra-vesicular noradrenaline is selectively localized in a site accessible via the membrane amine transport mechanism.

It is tempting to speculate that reversal of guanethidine-induced adrenergic neuronal blockade by sympathomimetic amines may be due to displacement of the blocker from an intraneuronal binding site. Reversal of adrenergic neuronal blockade has been shown to be accompanied by a loss of blocker from adrenergically innervated tissue (Kirpekar & Furchgott, 1972; Shand *et al.*, 1975). Although inhibitors of the neuronal membrane transport mechanism (e.g. cocaine) have been found to reverse adrenergic neuronal blockade, they appear to do so more gradually than amphetamine (Gerkins, McCulloch & Wilson, 1969) and are apparently more effective in preventing blockade than reversing it (Day, 1962) and do not reverse blockade persistently (Kirpekar, Wakade, Dixon & Prat, 1969). These observations suggest that while amphetamine may owe part of its adrenergic neuronal blockade reversal properties to its ability to prevent reuptake of adrenergic neuronal blockers, a major mechanism might be the displacement of the neurone blocker from intraneuronal binding sites. The selective displacement of adrenergic neuronal blocking drugs from a critical intraneuronal site might then allow such a store to refill with noradrenaline and thus restore nerve function. Some workers have concluded that adrenergic neurone blockers inhibit transmission by depressing the excitability of adrenergic nerve terminals (Haeusler, Haefely & Huerlimann, 1969). This interpretation does not account for the immediate and complete reversal of adrenergic blockade observed after administration of amphetamine and related compounds. Binding of adrenergic neurone blocking agents to a selective intraneuronal site might explain the rapid onset of nerve blockade as well as the rapid reversal seen after amphetamine. Displacement by amphetamine of bound [^{14}C]-guanethidine noted in this investigation has also been recently reported to occur for [^{3}H]-bretylium accumulated in rat atria (Ross & Gosztonyi, 1975). The role of extravesicularly bound noradrenaline in adrenergic transmission is speculative at the present. Evidence that the depolarization of neuronal membrane by nerve impulses promotes the release of noradrenaline from storage vesicles is firmly established (Malmfors, 1969; Farnebo, 1971) but little is known about the intermediate steps involved in this process.

It is possible that membrane depolarization activates a small intraneuronal pool of noradrenaline located near the terminal membrane; the liberation of noradrenaline from this site triggers the release of larger amounts of transmitter from storage vesicles. This concept is analogous to the mechanism postulated for acetylcholine in ganglionic transmission (Koelle, 1962); the adrenergic neurone would then utilize one of its characteristic constituents, i.e. noradrenaline in a sequential fashion to transmit information. Substitution of noradrenaline bound at the extravesicular site by guanethidine would result in suppression of transmitter release from storage vesicles. Day & Rand (1963), after examining the interactions of guanethidine and dexamphetamine concluded that these agents act competitively at 'receptors' located at the 'store' of noradrenaline. Our results support this interpretation and suggest that the 'receptors' alluded to are extravesicular binding sites in the adrenergic neurones.

This work was supported by U.S.P.H.S. Grant MH–05831, HL 19300–01.

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(Received February 14, 1976. Revised May 25, 1976.)