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Subcellular distribution of [^{3}H]amphetamine and [^{3}H]guanethidine and their interaction with adrenergic neurons

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The subcellular distribution of [^{3}H]amphetamine and [^{3}H]guanethidine and their interaction with each other and with noradrenaline binding sites have been examined. The ratio $p/(p + s) \times 100$, an indication of affinity for noradrenaline storage particles, for [^{3}H]amphetamine and [^{3}H]guanethidine was 12% and 57% respectively. Protriptyline, a substance which inhibits amine transport mechanism at the level of the cell membrane, i.e. the membrane pump, and reserpine, an agent which impairs incorporation of amines into the storage particles in the adrenergic nerve fibre, inhibited the uptake and storage respectively, of [^{3}H]guanethidine more than that of [^{3}H]amphetamine. Retention of [^{3}H]guanethidine by rat salivary glands was markedly decreased by sympathetic denervation of the glands while that of [^{3}H]amphetamine was not. The results suggest that guanethidine possesses a much higher affinity for noradrenaline binding sites than amphetamine.

A NUMBER of investigators (Laurence & Rosenheim, 1960; Day, 1962; Day & Rand, 1963; Matsumoto & Horita, 1963; Chang, Costa & Brodie, 1965; Brodie, Chang & Costa, 1965; Obianwu, 1967) have examined the ability of amphetamine-like compounds to antagonize the adrenergic nerve blockade produced by guanethidine. Since amphetamine can impair the tissue binding of guanethidine, it was suggested that amphetamine owes its antagonizing effects to a competition with guanethidine for noradrenaline storage sites and displacement of guanethidine from these sites (Day & Rand, 1963; Chang & others, 1965; Brodie & others, 1965). However, the observation that the antagonism of guanethidine binding is shared by several compounds, some of which have little or no ability to reverse guanethidine-induced nerve blockade (Brodie & others, 1965) suggests that the two processes may not be causally linked.

Little is known of the subcellular actions of these compounds. The experiments reported here were performed to study the subcellular distribution of guanethidine and amphetamine and to examine the interactions of these two compounds both with each other and with noradrenaline binding sites.

Experimental

SUBCELLULAR DISTRIBUTION

Mice, divided into groups of 6, were given either [^{3}H]guanethidine monosulphate, 200 μg/kg, or [^{3}H]-(+)-amphetamine sulphate, 20 μg/kg, intravenously. Some groups were used as controls while others received either reserpine, protriptyline, amphetamine (unlabelled) or guanethidine (unlabelled). For details of the injection schedule and doses see Results. At appropriate intervals the animals were killed by decapitation and their hearts homogenized in 0·25M sucrose containing 0·005M phosphate

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buffer, pH 7·4, and 0·001M $MgCl_2$. A coarse fraction was removed by centrifugation at 4° at 2000g for 10 min. The supernatant was then centrifuged at 100,000g for 60 min in a Spinco model L Ultracentrifuge, providing two more fractions, particulate (sediment) and high speed supernatant. After protein precipitation the fractions were assayed for their tritium content according to the procedures described below.

SYMPATHETIC DENERVATION OF THE SALIVARY GLAND

Male Sprague-Dawley rats, weighing about 200 g, were anaesthetized with hexobarbitone sodium (150 mg/kg, i.p.) and the right superior cervical ganglion was excised. In most of the animals the left jugular vein was permanently intubated (Popovic & Popovic, 1960) for intravenous injections. The animals were used 8–10 days after the operation.

ESTIMATION OF [^{3}H]-(+)-AMPHETAMINE AND [^{3}H]GUANETHIDINE

Tissues or fractions to be assayed were homogenized in 10 ml of ice cold 0·4N perchloric acid and then centrifuged in the cold at 2000g for 10 min. The extracts were filtered and then frozen at −25° until analysed. This was usually done within 3 days. [^{3}H]Amphetamine and [^{3}H]guanethidine were isolated by a column chromatography procedure similar to that described by Bertler (1961) and Andén & Magnusson (1967). The resin (Amberlite CG 50, type 1, 100–200 mesh) was previously prepared by being washed several times with dilute HCl and then transferred to 70 mm long glass tubes having an internal diameter of 4·5 mm. The height of the resin column was 45 mm. The column was then washed successively with 20 ml of 2N HCl, redistilled water until neutral (about 10 ml), 20 ml of 0·1M phosphate buffer pH 6·5 and 3 ml of redistilled water. After the above washes the resin had a length of 50 mm. The neutralized extract (pH 6·5) was then applied to the column at a rate not exceeding 0·5 ml per min (about 1 drop every 7 sec). After passage of the extract through the column it was washed with 10 ml of redistilled water.

Amphetamine was eluted with 18 ml of 0·5N acetic acid, the first 3 ml being discarded. Guanethidine was eluted with 19 ml of 0·1N HCl, the first 5 ml being discarded. Since the rate of elution was usually increased after the passage of a few ml of acid, it was necessary to re-adjust the flow to 0·5 ml/min. The eluates were freeze-dried and 5 ml of scintillation mixture (9 parts of toluene containing 3 g PPO and 0·3 g POPOP per litre, and 1 part of ethanol containing 1% conc. HCl) were added and the tritium content determined by liquid scintillation counting.

Mean recovery values of known amounts of [^{3}H]guanethidine or [^{3}H]amphetamine added to tissue extracts were 85 and 90%, respectively. No corrections were made for these recoveries.

IDENTIFICATION OF ISOLATED [^{3}H]GUANETHIDINE AND [^{3}H]-(+)-AMPHETAMINE

Two groups of mice were injected with [^{3}H]guanethidine and [^{3}H]-(+)-amphetamine respectively and killed 1 hr later. The hearts were removed and the tritiated compounds were isolated as described above.

IDENTIFICATION OF ISOLATED [^{3}H]AMPHETAMINE

The freeze-dried eluate was dissolved in a few drops of redistilled water and subjected to paper chromatography. The solvent system used was butanol–acetic acid–water (4 : 1 : 5) and gave a single peak of radioactivity with an Rf value of 0·87 which was identical to that of authentic [^{3}H]amphetamine. Radioactivity was measured by counting 1 cm strips of the chromatogram as described above.

IDENTIFICATION OF ISOLATED [^{3}H]GUANETHIDINE

The freeze dried eluate was dissolved in a few drops of redistilled water and subjected to paper chromatography using two solvent systems, isopropanol–conc. ammonia–water (20:2:3, 16 hr) and butanol–ethanol–10% ammonium hydroxide (40:20:13, 16 hr). The chromatograms showed single peaks of radioactivity with Rf values of 0·76 and 0·62, respectively. These corresponded to the peaks obtained using authentic [^{3}H]guanethidine.

ESTIMATION OF ENDOGENOUS NORADRENALINE

Endogenous noradrenaline content of cardiac tissue was measured by the method of Bertler, Carlsson & Rosengren (1958).

DRUGS

[^{3}H]Guanethidine (2-(octahydro-1-azocinyl)-1-[^{3}H]ethyl guanidine) monosulphate, specific activity 51·9 μc/mg, was kindly supplied by Dr. C. I. Furst of CIBA Laboratories Ltd. Horsham, Sussex, England. [^{3}H]-(+)-Amphetamine sulphate, specific activities of 8·9 c/mmole and 4·23 c/mmole, generally labelled, were obtained from New England Nuclear Corporation. Other drugs and their suppliers are as follows: reserpine (Serpasil) and guanethidine (Ismelin), Ciba Ltd.; protriptyline, Dr. C. A. Stone of the Merck Institute for Therapeutic Research; and (+)-amphetamine bitartrate commercially obtained. The doses refer to the bases of the compounds.

Results

SUBCELLULAR DISTRIBUTION OF [^{3}H]GUANETHIDINE IN THE MOUSE HEART

The subcellular distribution of [^{3}H]guanethidine 0·5, 1 and 4 hr after its intravenous administration is shown in Table 1. After 0·5 hr [^{3}H]-guanethidine was found in all subcellular fractions of the heart. The amount of [^{3}H]guanethidine retained in the coarse fraction was more than twice that retained by either the supernatant or particulate fraction. During the 4 hr interval after its injection, there was an increase in both the absolute and relative amounts of [^{3}H]guanethidine found in the particulate fraction, and a concomitant decrease in the amount of tritiated material found in the supernatant fraction. There was no appreciable decrease in the total amount of [^{3}H]guanethidine that could be recovered from the heart during this 4 hr period.

TABLE 1. SUBCELLULAR DISTRIBUTION OF [^{3}H]GUANETHIDINE AND [^{3}H]-(+)-AMPHETAMINE IN THE MOUSE HEART. Mice were given [^{3}H]guanethidine, 200 μg/kg, i.v. or [^{3}H]amphetamine, 20 μg/kg, i.v. and were killed at various intervals. The values are in ng/g tissue ± s.e. Six hearts were pooled for each determination. When the tritiated compounds were added directly to heart tissues before homogenization, the p/(p + s) ratio for guanethidine and amphetamine were 15·5 and 3·4 respectively.

Drug	Interval in min	Coarse (c)	Particulate (p)	Supernatant (s)	$\frac{p}{p+s} \times 100$	Total	No. of experiments
[^{3}H]guanethidine	30	147·99 ± 29·97	61·76 ± 5·25	62·85 ± 6·00	49·64 ± 0·68	272·60 ± 36·62	4
	60	141·53 ± 23·04	73·01 ± 8·26	55·93 ± 10·63	57·28 ± 3·49	270·47 ± 38·04	4
	240	174·27 ± 7·17	84·19 ± 6·63	41·74 ± 7·51	67·68 ± 3·85	300·21 ± 17·86	6
[^{3}H]amphetamine	30	1·63 ± 0·16	0·31 ± 0·03	5·41 ± 0·34	5·39 ± 0·34	7·35 ± 0·04	4
	60	0·88 ± 0·11	0·28 ± 0·02	2·10 ± 0·07	12·09 ± 0·35	3·26 ± 0·05	3

TABLE 2. EFFECT OF PROTRIPTYLINE AND RESERPINE ON THE SUBCELLULAR DISTRIBUTION OF [^{3}H]GUANETHIDINE AND [^{3}H]AMPHETAMINE. Mice were pretreated with protriptyline, 10 mg/kg, i.p. half hr or reserpine, 5 mg/kg, i.p. 4 hr before administration of [^{3}H] guanethidine, 0·2 mg/kg, i.v. or [^{3}H]amphetamine, 20 μg/kg, i.v. The animals were killed 1 hr after injection of the tritiated compounds. Control animals were not pretreated with protriptyline or reserpine. Six hearts were pooled for each determination and the values, in ng/g tissue, are means of four determinations ± s.e.

Drug	Treatment	Coarse	Significance test	Particulate	Significance test	Supernatant	Significance test	$\frac{p}{p+s} \times 100$	Total
[^{3}H]guanethidine	Control	127·46 ± 14·28		59·27 ± 2·77		44·99 ± 4·44		57·11 ± 1·86	231·72 ± 10·95
	Protriptyline	26·93 ± 3·87	$P<0·001$	4·41 ± 0·34	$P<0·001$	24·68 ± 3·72	$P<0·025$	15·93 ± 2·51	56·02 ± 7·01
	Reserpine	57·00 ± 6·60	$P<0·005$	15·08 ± 1·10	$P<0·001$	94·54 ± 13·93	$P<0·025$	14·20 ± 1·38	166·62 ± 23·35
[^{3}H]amphetamine	Control	0·88 ± 0·11		0·28 ± 0·02		2·10 ± 0·07		12·09 ± 0·34	3·26 ± 0·05
	Protriptyline	0·55 ± 0·04	$P<0·05$	0·12 ± 0·01	$P<0·001$	2·62 ± 0·06	$P<0·01$	4·41 ± 0·06	3·30 ± 0·05
	Reserpine	0·76 ± 0·01	$P>0·25$	0·12 ± 0·05	$P<0·001$	2·42 ± 0·04	$P=0·05$	4·86 ± 0·04	3·31 ± 0·04

SUBCELLULAR DISTRIBUTION OF [^{3}H]-(+)-AMPHETAMINE IN THE MOUSE HEART

The concentrations of [^{3}H]-(+)-amphetamine found in various fractions of the mouse heart are shown in Table 1. After 0·5 hr the highest concentration of tritiated amine was found in the supernatant fraction while the lowest levels were found in the particulate fraction. One hr after its administration, levels of [^{3}H]-(+)-amphetamine, unlike [^{3}H]guanethidine, showed a decrease in the amount retained by all subcellular fractions. This resulted in a net decrease (about 55%) of [^{3}H]amphetamine from the heart. The decrease in [^{3}H]amphetamine concentrations resulted in an increase in the p/(p + s) ratio. This appeared to be due to a more rapid loss of [^{3}H]-(+)-amphetamine from the supernatant (s) than the particulate fractions (p).

EFFECT OF PROTRIPTYLINE ON THE ACCUMULATION OF [^{3}H]GUANETHIDINE and [^{3}H]-(+)-AMPHETAMINE

Protriptyline markedly decreased the retention of [^{3}H]guanethidine in all subcellular fractions, the decrease being greatest in the particulate fraction. There was a decrease of about 75% in the total amount of [^{3}H]guanethidine found in the heart (Table 2).

Pretreatment with protriptyline produced a moderate decrease in the retention of [^{3}H]-(+)-amphetamine in the coarse and particulate fractions and slightly increased the amount found in the supernatant fraction. There was, however, practically no change in the total amount of [^{3}H]-(+)-amphetamine found in the heart after protriptyline administration (Table 2).

EFFECT OF RESERPINE ON THE SUBCELLULAR DISTRIBUTION OF [^{3}H]GUANETHIDINE AND [^{3}H]-(+)-AMPHETAMINE

Reserpine pretreatment greatly inhibited the retention of [^{3}H]guanethidine in the coarse and particulate fractions and appreciably increased the amount found in the supernatant (Table 2). The retention of [^{3}H]amphetamine was only impaired in the particulate fraction (Table 2). The slight increase in the amount of tritiated amine in the supernatant fraction was not statistically significant (Table 2). Reserpine more markedly reduced the p/p + s ratio for guanethidine (57 to 14%) than it did for amphetamine (12 to 5%).

EFFECT OF SYMPATHETIC DENERVATION ON THE UPTAKE AND BINDING OF [^{3}H]GUANETHIDINE AND [^{3}H]-(+)-AMPHETAMINE

The retention of [^{3}H]guanethidine and [^{3}H]-(+)-amphetamine was measured 8–10 days after unilateral denervation of the rat salivary gland (Table 3). One-half hr after the administration of [^{3}H]guanethidine the amount retained in the innervated gland was significantly ($P < 0·05$) greater than that retained in the denervated gland. This difference increased with time after injection of the labelled substance. There was about 60% less [^{3}H]guanethidine in the denervated gland 6 hr after its

administration. [^{3}H]-(+)-amphetamine, on the other hand, was not preferentially retained by the innervated gland. In fact, the amount of [^{3}H]amphetamine retained by the denervated gland appeared to be higher than that found in the innervated organ (Table 3).

TABLE 3. EFFECT OF SYMPATHETIC DENERVATION ON THE UPTAKE AND BINDING OF [^{3}H]guanethidine and [^{3}H]amphetamine. Eight to ten days after chronic unilateral denervation of rat submaxillary glands the animals were given [^{3}H]guanethidine, 200 μg/kg, i.v. or [^{3}H]amphetamine 5 μg/kg, i.v. and were killed at various intervals. The values are in ng/g tissue and are means of three to four determinations ± s.e.

	[^{3}H]Guanethidine			[^{3}H]Amphetamine		
Interval	Innervated gland	Denervated gland	Significance test	Innervated gland	Denervated gland	Significance test
30 min	524 ± 57	377 ± 18	P < 0·05	11·79 ± 1·19	15·09 ± 4·22	P > 0·25
60 min	463 ± 26	301 ± 30	P < 0·005	7·21 ± 1·96	14·01 ± 1·70	P > 0·05
3 hr	385 ± 48	140 ± 22	P < 0·005	2·52 ± 0·22	5·93 ± 1·54	P > 0·1
6 hr	283 ± 8	108 ± 18	P < 0·001	—	—	—

INTERACTION OF GUANETHIDINE AND AMPHETAMINE WITH THEIR RESPECTIVE BINDING SITES

Guanethidine (15 mg/kg) pretreatment markedly inhibited the accumulation of [^{3}H]-(+)-amphetamine in all subcellular fractions (Table 4). This inhibitory action of guanethidine was more pronounced than that of either protriptyline or reserpine. Pretreatment of mice with unlabelled amphetamine (1 mg/kg) resulted in a decrease in the amount of [^{3}H]guanethidine retained in the heart. This impairment of retention or binding was most pronounced in the coarse and particulate fractions while the amount of [^{3}H]guanethidine found in the supernatant fraction was not significantly altered. The inhibitory effect of amphetamine appeared to be less pronounced than that produced by either protriptyline or reserpine.

EFFECT OF AMPHETAMINE ON GUANETHIDINE-INDUCED DEPLETION OF CARDIAC NORADRENALINE STORES

Guanethidine in a dose of 15 mg/kg, intraperitoneally, caused a decrease of heart noradrenaline of about 86% in 6 hr (Table 5). Treatment with amphetamine, 1 mg/kg intraperitoneally, 0·5 hr before or after guanethidine administration appeared to partially antagonize the depletion of heart noradrenaline by guanethidine. However, only in the latter case was the antagonistic effect statistically significant.

Discussion

Indirect evidence suggests that guanethidine may be retained in sympathetic nerves at sites which are similar or identical to those storing noradrenaline. Both noradrenaline and reserpine can reduce the uptake of guanethidine into sympathetically innervated tissue, and reserpine can release [^{3}H]guanethidine already accumulated (Chang, Costa & Brodie,

TABLE 4. INTERACTION OF GUANETHIDINE AND AMPHETAMINE WITH NORADRENALINE STORAGE SITES. Mice were pretreated with guanethidine, 15 mg/kg, i.p. or amphetamine, 1 mg/kg, i.p. half hr before injection of [^{3}H]amphetamine, 20 μg/kg, i.v. or [^{3}H]-guanethidine, 200 μg/kg, i.v. and were killed 1 hr after injection of the tritiated compounds. The values, in ng/g tissue, are means of four determinations ± s.e. Six hearts were pooled for each determination.

Drug	Treatment	Particulate	Significance test	Supernatant	Significance test	$\frac{p}{p+s} \times 100$	Total
[^{3}H]Guanethidine	None	59·27 ± 2·77	—	44·99 ± 4·50	—	57·11 ± 1·86	231·72
	Amphetamine	27·92 ± 2·44	P > 0·001	35·76 ± 4·36	P > 0·1	44·15 ± 2·92	122·45 ± 6·87
[^{3}H]Amphetamine	None	0·28 ± 0·02	—	2·10 ± 0·07	—	12·09 ± 0·34	3·26 ± 0·05
	Guanethidine	0·08 ± 0·03	P < 0·001	1·38 ± 0·08	P < 0·005	5·60 ± 0·30	1·74 ± 0·05

TABLE 5. EFFECT OF AMPHETAMINE ON THE GUANETHIDINE-INDUCED DEPLETION OF HEART NORADRENALINE IN MICE. Mice were given guanethidine, 15 mg/kg, i.p. half hr before or after amphetamine, 1 mg/kg, i.p. The animals were killed six hr after injection of guanethidine. Six hearts were pooled for each determination.

Treatment	Heart noradrenaline in μg/g tissue	No. of experiments
None	0·634 ± 0·022	4
Guanethidine	0·086 ± 0·005	4
Amphetamine	0·588 ± 0·066[1]	3
Guanethidine + Amphetamine	0·156 ± 0·011[2]	4
Amphetamine + Guanethidine	0·106 ± 0·011[3]	4

[1] P > 0·25 compared to control.
[2] P < 0·005 compared to guanethidine-treated animals.
[3] P > 0·1 compared to guanethidine-treated animals.

1964; Chang & others, 1965). Furthermore, guanethidine can be released by sympathetic nerve stimulation (Boullin, Costa & Brodie, 1966), and has been shown to have an effect on both the sympathetic nerve cell membrane pump and on an intracellular catecholamine-concentrating mechanism (Lindmar & Muscholl, 1964; Shore & Giachetti, 1966; Lundborg & Stitzel, 1967). The above observations prompted us to examine in more detail the distribution of this compound in sympathetic tissue and to examine its interaction with amphetamine, a compound known to antagonize the adrenergic nerve blockade produced by guanethidine (for references see introduction).

It has been shown that compounds such as metaraminol and α-methylnoradrenaline as well as noradrenaline can be taken up by and stored in a compartment isolated during a subfractionation of cardiac tissue (Lundborg & Stitzel, 1967). This compartment or fraction apparently contains particles which normally bind noradrenaline within the sympathetic neuron (cf. Potter, 1966). The above experiments, which employed a fractionation procedure similar to the one reported here indicated that [^{3}H]noradrenaline, [^{3}H]α-methylnoradrenaline and [^{3}H]metaraminol were present in a p/(p + s) ratio (i.e. the amount of labelled compound in the particulate fraction, expressed as a percentage of that found in the particulate + supernatant fractions) of 48, 37 and 19% respectively 1 hr after their intravenous administration.

The present studies indicate that a high proportion of the [^{3}H]guanethidine taken up by the heart is also associated with the particulate fraction. One hr after its administration the p/(p + s) ratio was about 56%. Thus guanethidine appears to possess a high affinity for the noradrenaline containing particulate fraction. [^{3}H]amphetamine, on the other hand, does not appear to possess nearly as high an affinity for these noradrenaline storage sites. One hr after its administration the p/(p + s) ratio for amphetamine was only about 12%, indicating that, in the doses used here, guanethidine is much more tightly bound to the particulate fraction than amphetamine.

Evidence supporting the existence of an amine-concentrating mechanism at the level of the neuronal cell membrane has been presented (Malmfors, 1965; Carlsson & Waldeck, 1965a). Protriptyline appears to be a potent inhibitor of this uptake mechanism (Carlsson & Waldeck, 1965b). Mice pretreated with protriptyline showed a greatly impaired ability to accumulate [^{3}H]guanethidine. Both the total uptake and the uptake into subcellular fractions were markedly decreased. This suggests that most of the [^{3}H]guanethidine taken up by the heart is (1) located intraneuronally and that (2) [^{3}H]guanethidine probably utilizes the membrane pump for its initial accumulation within the sympathetic neuron. Although protriptyline caused a decrease in the concentration of [^{3}H]-(+)-amphetamine found in the particulate fraction, the total amount of [^{3}H]-(+)-amphetamine taken up in the heart did not differ from that of non-protriptyline-treated mice. Apparently [^{3}H]-(+)-amphetamine is accumulated by the membrane pump concentrating mechanism to only a limited degree.

Support for such a conclusion is found in the recent work of Ross & Renyi (1966) who reported that [^{3}H]-(+)-amphetamine was accumulated in tissues by a non-specific process and that this accumulation could not be reduced by neuronal membrane pump inhibitors.

Chronic sympathetic denervation of rat submaxillary gland markedly inhibited accumulation of [^{3}H]guanethidine by this organ, but not that of [^{3}H]-(+)-amphetamine. However, caution should be exercised in interpreting this result since denervation appeared to increase the amount of [^{3}H]amphetamine retained by the denervated gland. The relatively large amount of [^{3}H]guanethidine retained by the denervated gland probably indicates that the denervation was not complete. It may also reflect unspecific binding of guanethidine since this substance appears to be retained at both specific (noradrenaline binding sites) and unspecific sites (Chang & others, 1965).

Our studies of the influence of amphetamine pretreatment on guanethidine-induced depletion of heart noradrenaline were not conclusive. While amphetamine administered 30 min before guanethidine did not significantly influence depletion of heart noradrenaline, the same dose of amphetamine given 30 min after guanethidine did partially antagonize the depletion of heart noradrenaline.

Reserpine, a compound known to both prevent the uptake of exogenous noradrenaline into noradrenaline storage granules (Carlsson, Hillarp & Waldeck, 1962; Kirshner, 1962) and to alter its subcellular distribution (Stitzel & Lundborg, 1967) had less effect on amphetamine uptake and distribution than it did on guanethidine. After reserpine pretreatment the amount of guanethidine found in the particulate fraction was markedly reduced with a corresponding increase in the amount of drug found in the supernatant fraction. Less amphetamine was also found in the particulate fraction but without a corresponding increase in the supernatant. However, some degree of competition between guanethidine and amphetamine does seem to exist. Although only a relatively small amount of injected [^{3}H]-(+)-amphetamine taken up by the heart is retained by the particulate fraction, its retention can be impaired by pretreatment with guanethidine (15 mg/kg), and pretreatment with amphetamine (1 mg/kg) can partially impair [^{3}H]guanethidine accumulation.

The present studies indicate that guanethidine has a much higher affinity for noradrenaline binding sites than amphetamine and that procedures which impair uptake and binding of noradrenaline inhibit uptake and binding of guanethidine to a greater extent than those of amphetamine.

Although our studies indicate that the two substances do not generally compete for the same binding sites, limited competition for binding at functional storage sites (i.e. sites from which noradrenaline is released by nerve impulse) cannot be ruled out.

The mechanism of the nerve blockade reversal brought about by amphetamine is still unclear, though a competition for common binding sites or a displacement of guanethidine by amphetamine, or both, has been

proposed. It is known that the adrenergic nerve blockade induced by guanethidine is associated with its ability to prevent the release of noradrenaline from the sympathetic nerve ending. Amphetamine may exert its antagonistic action by facilitating the release of the transmitter by increasing the permeability of the membrane. Previous reports that amphetamine potentiates responses to nerve stimulation (Ryall, 1961; Day & Rand, 1963; Dóda, György & Nádor, 1966; Obianwu, 1967) are consistent with this view.

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