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H. STORAGE OF NOREPINEPHRINE IN SYMPATHETIC NERVES

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In 1953 Blaschko and Welch (2) and Hillarp *et al.* (34) deomonstrated that epinephrine (E) in homogenates of the adrenal medulla was localized in large particles. These granules were subsequently identified as chromaffin granules, and carefully studied (31). In 1956 von Euler and Hillarp (12) used similar centrifugation techniques to study the storage of norepinephrine (NE) in the splenic nerves and spleen of oxen and the spleen of rats; they demonstrated that some of the NE stored in these tissues could be isolated from homogenates in subcellular particles. Since that time a great deal of information has been obtained about the character, properties and function of such granules.

IDENTIFICATION OF VESICLES WHICH STORE NE IN SYMPATHETIC NERVES

Electron microscopy reveals the presence of several discrete types of vesicle in tissues of neural origin (table 1). Of these particles only "small granulated vesicles" have been shown to be associated with NE. Five separate lines of evidence may be considered. 1) Small and large granulated vesicles and synaptic vesicles are evident in autonomic axons in the normal dog heart, but small granulated vesicles could not be found in the nerves remaining in transplanted hearts when NE was absent, whereas the other vesicles remained (56). 2) A particulate fraction containing NE has been isolated from the rat heart; it consists largely of vesicles 50 mμ in diameter some of which have a dense core (discussed below). 3) Electron micrographs of the iris of the albino rabbit show many small granulated vesicles in the nerves of the dilator muscle, which is innervated predominantly by adrenergic axons; whereas synaptic vesicles are characteristic of the nerves of the sphincter muscle, which is innervated predominantly by cholinergic nerves (59). 4) The dense cores of small granulated vesicles in the nerves of the smooth muscle of the rat vas deferens disappear following a single large dose of reserpine and then reappear, and the time course and degree of their depletion are closely correlated with the disappearance and reappearance of NE (Richardson and Potter, unpublished; *cf.* 57). 5) Combined electron microscopy and radioautography of tissues containing H^3-NE shows silver grains only in association with regions of nerve axons containing small granulated vesicles (71, 70). These studies emphasize that small granulated vesicles must be carefully distinguished from other particles present in nerves.

ISOLATION OF VESICLES STORING NE

Euler and his coworkers (9) and Schümann (61) have shown that particles containing NE can readily be obtained from the midportion of adrenergic axons within bovine splenic nerve trunks. These axon bundles, when desheathed, contain up to 20 μg of NE per g. Particles have been obtained by squeezing out

TABLE 1

Vesicles in cells of neural origin

Vesicle	Diameter	Distribution	Probable Content
"Synaptic vesicles"	*ca.* 50 mμ	Most central and peripheral nerve endings	Considered characteristic of chemical synapses; some in brain contain acetylcholine (58, 69)
"Small granulated vesicles"	*ca.* 50 mμ core 20–30 mμ	Postganglionic adrenergic axons	Norepinephrine (see text)
"Large granulated vesicles"	80–100 mμ core 40–50 mμ	Many central and peripheral axons, especially preganglionic nerve terminals (30)	Unknown
"Neurosecretory granules"	90–200 mμ core variable	Secreting neurons, *e.g.* supraoptic nucleus (49)	*E.g.*, neurohypophyseal hormones
"Chromaffin granules"	50–500 mμ core variable	Chromaffin cells	Catecholamines (31)

"press juice" from the nerves between nylon rollers, and phosphate buffers (0.13 M, pH 7.5) have generally been used to dilute the suspension obtained (15). Such suspensions have been intensively studied after removal of large particles by centrifugation at 600 to 1000 × *g* for 10 min. A maximal yield of storage granules may be obtained by centrifuging the suspension at 50,000 × *g* for 30 min (10). The pellets obtained are nearly colorless and translucent and contain an average of 28% of the NE in the "press juice" (22). Recent micrographs of phosphotungstate-stained particles obtained by these methods show vesicles 30 to 100 mμ in diameter displaying prominent "notching" (27). As yet a comparison of these particles with vesicles normally present in splenic nerves has not been published.

Isolation of small granulated vesicles from sympathetic nerve endings and preterminals has proved difficult. By 1961 NE had been reported in particles from two tissues, the spleen (12) and puppy hearts (67). At that time electron micrographs of adrenergic nerves were not generally available and the results obtained by Euler and Schümann suggested that NE storage particles could be sedimented like chromaffin granules (*e.g.*, at 9,000 × *g* for 10 min). In 1962 it was found unexpectedly that particles obtained from homogenates of the rat heart, containing endogenous and exogenous (H^3-labeled) NE, sedimented during gradient centrifugation with particles of "microsomal" size (50). Similar results were obtained for endogenous and H^3-NE in the salivary gland and vas deferens,

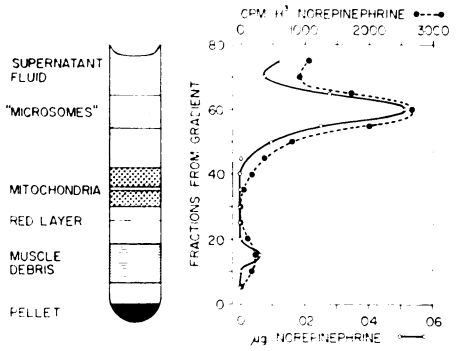

FIG. 1. Subcellular localization of H^3-*l*-NE and endogenous NE in the rat heart.

An albino rat weighing 150 g was given 50 μc H^3-*l*-NE (1650 mC/mmol) intravenously, and its heart was removed and homogenized in 2.5 ml of 0.25 M sucrose, 30 min later. One milliliter of the homogenate was centrifuged over a continuous density gradient of sucrose (0.25–2.2 M) at 125,000 × *g* for 30 min. Preparations, samples and assays were as described by Potter and Axelrod (51). The figure shows the appearance of the gradient after centrifugation, and the distribution of the amines in the gradient. The pellet contained 3312 cpm H^3-NE and 0.04 μg NE.

and for H^3-NE in the pineal and adrenal glands (51). Isolated microsomal particles did not sediment in isotonic buffer solutions or sucrose at 9,000 × *g* for 10 min. However, "trapping" of the NE vesicles frequently occurred in the mitochondrial and other easily sedimented particle fractions during differential centrifugation of whole homogenates (51). Similar findings were obtained by Gillis (28) with the rabbit heart. Most subsequent investigators have used density gradient centrifugation to separate microsomes from larger particles (fig. 1) or have discarded particles which sediment in isotonic sucrose at 105,000 × *g* for 5 min (52), 12,800 × *g* for 15 min (29), or 20,000 × *g* for 10 min (47). These gravitational forces are sufficient to remove mitochondria (29, 55) but also lead to losses of the storage vesicles. A better method of removing large particles is centrifugation of homogenates in 0.7 M sucrose at 105,000 × *g* for 10 min, since the storage vesicles do not sediment under these conditions (Potter, unpublished).

Several attempts have been made to isolate the specific NE vesicles from other microsomal particles obtained from the heart. Modification of the original gradients (50, 51) to 0.25 to 1.0 M sucrose permitted the isolation of pellets composed of many clear vesicles 50 to 120 mμ in diameter, some ribonucleoprotein granules, occasional glycogen granules, and a few small granulated vesicles (55). Improved results were obtained by Michaelson *et al.* (43) using 0.25 to 0.73 M sucrose gradients, in that fewer ribosomes remained in the pellets studied by electron microscopy. The same preparations showed a 60-fold purification in terms of H^3-NE per μg protein, and a 40-fold increase in cpm/μg RNA (64). A recent improvement in technique has yielded pellets containing no detectable RNA and amounts of protein corresponding to a 1500-fold purification (Potter, unpublished). Rat heart tissue containing H^3-NE is homogenized in 0.25 M

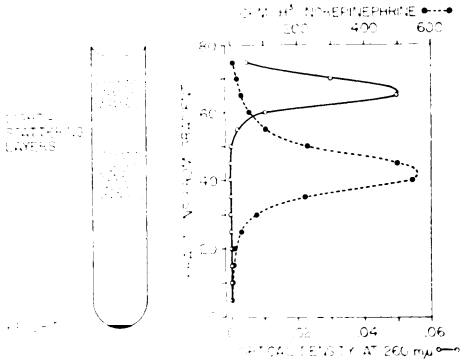

FIG. 2. Isolation of small vesicles containing H^3-NE from other "microsomal" particles obtained from the rat heart.

Two milliliters of the homogenate prepared for figure 1 were centrifuged at 105,000 × *g* for 5 min and the supernatant fluid and remaining microsomes were filtered through a 1 × 15 cm column of Sephadex G-200 gel. One milliliter of the column eluate was centrifuged over a sucrose gradient (0.25–1.0 M) at 125,000 × *g* for 60 min. Samples and assays were as described by Potter and Axelrod (51); absorption of light at 260 mμ is a measure of the nucleic acid present in ribosomes. The pellet contained 352 cpm H^3-NE.

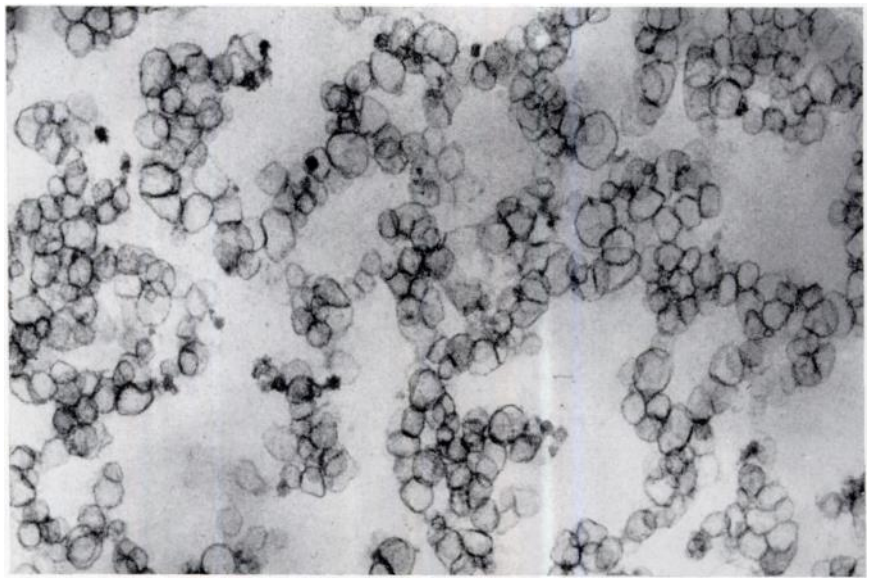

FIG. 3. Fine structure of isolated cardiac vesicles. Vesicles containing H^3-NE were removed from the sucrose gradient shown in figure 2, diluted with isotonic sucrose, and sedimented at 105,000 × *g* for 60 min. A tiny pellet was visible following fixation and staining with glutaraldehyde and osmium; it was studied with the electron microscope by Dr. D. Wolfe. The vesicles shown vary in diameter from about 30 to 200 mμ in diameter, and the granulated vesicle measures 60 mμ in diameter. Present evidence indicates storage of NE only in such small granulated vesicles.

sucrose and centrifuged at 105,000 × *g* for 5 min to remove large particles, including large vesicles and some ribosomes and glycogen granules. The supernatant fluid and remaining microsomes are filtered through Sephadex G-200 gel (bead-form, in sucrose without added ions) in order to remove soluble NE and most soluble proteins. This step avoids the trauma of sedimentation and rehomogenization of the particles, and in addition alters the ribonucleoprotein granules so that they do not subsequently sediment with the NE-containing vesicles. Finally gradient centrifugation (0.25 to 1.0 M sucrose) removes most of the remaining vesicles and ribosomes (fig. 2). An electron micrograph of the peak fraction in the gradient of figure 2 is shown in figure 3.

Artifacts in the preparation of these vesicles do not contribute significantly to the results obtained since less than 4% of H^3-NE homogenized with tissues becomes adsorbed to microsomal particles at 4°C (51, 52). The small amounts of H^3-NE which are briefly bound at unknown sites in transplanted hearts appear upon subcellular fractionation only in soluble form (56).

PHYSICAL PROPERTIES OF NE STORAGE VESICLES

The physical properties of the splenic nerve vesicles which contain NE (15, 65) and of the rat heart vesicles which retain endogenous and labeled NE (52) have been well studied and are very similar (table 2). Both are lysed by detergents, but are moderately resistant to lysis by suspension in water [like synaptic vesicles (58, 69) and unlike chromaffin granules (35)] or by freezing and thawing. Both rapidly lose NE in warm solutions, in which adrenal granules are much more stable. Both vesicle preparations retain NE against great gradients: the splenic particles resist the action of oxidizing agents, and remain intact when stirred or filtered through alumina at pH 8.2; and the heart vesicles have been dialyzed for 4 hr, or passed repeatedly through Sephadex gels without loss of NE. Stjärne (65) found, however, that splenic nerve vesicles are apparently permeable to NE even at 0°C, a circumstance that permitted exchange of H^3-NE with endogenous NE in the vesicles.

BIOCHEMICAL PROPERTIES OF NOREPINEPHRINE STORAGE VESICLES

No preparation of vesicles containing NE has yet been obtained sufficiently pure to permit accurate measurement of the concentrations of lipids, proteins, adenine nucleotides and other constituents of the vesicles, comparable to the measurements in isolated adrenal chromaffin granules by Hillarp (53). The average content of NE per granulated vesicle is not known. However the presence or absence of several enzymes in partially purified preparations of heart vesicles and the presence of ATP in both splenic nerve and heart vesicles has been demonstrated (see table 3 for references). Tyrosine hydroxylase could not be detected in heart microsomes, although it was present in mitochondria. Dopa decarboxylase is present in small heart vesicles in higher yield than any other apparently soluble enzyme studied, but the functional significance of this finding is not now clear. Dopamine-β-hydroxylase, which converts dopamine to NE, is present in the membranes of adrenal chromaffin granules (41). The enzyme is also detectable

TABLE 2

Physical properties of NE storage vesicles

Variable	Stability of NE in Splenic Nerve Vesicles	Stability of H^3-NE in Cardiac Nerve Vesicles
Solution (isotonic)	Similar in K-phosphate, sucrose, $CaCl_2$, NaCl, KCl, $MgCl_2$ and Na-acetate (15)	Decreasing stability: $CaCl_2$, sucrose, $MgCl_2$, KCl, NaCl, "Krebs", K-phosphate, Na-acetate, "Tris" (52)
pH	Stable pH 5–8, slight losses at 9, lysis below 4 (15)	Same (52)
Tonicity	About 40% release in H_2O or 1.5 M KCl in 5 min plus centrifugation time (15, 65)	60% release in H_2O for 30 min plus centrifugation time (52)
Temperature	No losses at 4°C in 3 hr	No losses at 4°C in 2 hr, 55% loss at 4°C in 24 hr (52)
	50% release in 60 min at 23°C or in 10 min at 37°C (15, 62, 65)	Same (52)
Detergents	1 mM cetyl pyridium Br or Na-lauryl sulfonate: 83–92% release (15)	0.1% deoxycholate: 18% release
	Saponin 30 mg/ml, or ether 1% no effect (15)	0.5% deoxycholate: 98% release (52)
Freezing and thawing (×6)	28% release (15)	36% release[a]
Oxidizing agents	NE not oxidized by manganese dioxide (15)	
Miscellaneous	EDTA: no effect	0.1 M pyrophosphate: 20% release
	Filtration or mixing with alumina at pH 8.2: no effect	No losses on filtration through Sephadex G-200 in sucrose (52)
		No losses on dialysis *vs.* sucrose 4 hr at 4°C[a]
		Intact particles inactive *in vivo* (52)

[a] Potter, unpublished.

in the membranes of small heart microsomes, but more work is necessary to confirm its presence only in the granulated vesicles within this preparation. It appears possible, however, that nerve storage vesicles can synthesize NE at least from dopamine, in addition to taking up the transmitter from the axoplasm. Only tiny amounts of phenylethanolamine-N-methyl transferase, which converts NE to E, are present in extrachromaffin tissues (1) and only traces of this enzyme are found in isolated heart particles which contain NE. Catechol-O-methyl transferase (COMT) and monoamine oxidase (MAO) are almost absent from heart vesicle preparations, a fact that indicates that the degradation of NE in nerves occurs after its release from storage vesicles.

TABLE 3

Biochemical properties of particles obtained from rat heart microsomes and bovine splenic nerves

Constituent	Determination
1. Enzymes of catecholamine synthesis:	
Tyrosine hydroxylase	Not detected in small heart microsomes[a]
DOPA decarboxylase	4% of heart enzyme in small microsomes[a]
Dopamine β-hydroxylase	Detectable in membranes of small heart microsomes (52)
Phenylethanolamine-N-methyl transferase	Less than 0.4% of cardiac enzyme in small microsomes[a]
2. Enzymes of catecholamine degradation:	
Catechol-O-methyl transferase	Less than 0.3% of cardiac enzyme in small microsomes (52)
Monoamine oxidase	Less than 0.3% of cardiac enzyme in small microsomes (52)
3. Other enzymes	
ATPase	Mg^{++} and Ca^{++}-activated ATPase present in purest heart vesicle preparations[a]
4. ATP	Present in splenic nerve particles in molar ratio (NE:ATP) about 4:1 (12, 61, 65)
	Present in partially purified cardiac vesicles in similar 4:1 ratio (52)

[a] Potter, unpublished.

ATP is present in remarkable quantities in adrenal chromaffin granules [15% dry weight (33)] and is found in comparable ratios to catecholamine in preparations of these granules and splenic nerve and heart vesicles. Since it appears to be moderately inert within the particles, and the above ratio is relatively constant (see 65 for review and important exceptions), ATP has often been suggested as an ionic complexing agent responsible for concentrating amines within vesicles or as the final binding substance, or both. Weak catecholamine-ATP complexes have been demonstrated in solutions (68). Some such complex of the amines with ATP, with ATP associated with proteins, or with proteins directly, is probable since the intravesicular concentration of amines, at least in adrenal granules [0.3 to 1.1 M (3)] would be hypertonic if present in free solution, and lead to osmotic lysis of the vesicles. Mg^{++}-activated ATPases are present in chromaffin granules (Banks and Blaschko, Section IV H) and both Mg^{++}- and Ca^{++}-activated ATPases occur in the best present preparations of heart vesicles which store NE. Since Ca^{++} are known to be essential for the secretion of catecholamines from the adrenal medulla (8), it is tempting to speculate that stimulus-secretion coupling involves an ion-activated enzyme, like ATPase, which either causes an abrupt disruption of a catecholamine binding complex or causes a sudden change in membrane permeability.

SPECIFICITY OF STORAGE VESICLES FOR NE; POSSIBLE BINDING MECHANISMS

Recent studies have demonstrated that the binding mechanisms for NE in storage vesicles are not specific for this amine (see table 4 for references). The following correlations between chemical structure and binding have been established. The quaternary N^+ group is essential for binding (dihydroxymandelic acid is not retained) and increasing N-substitution decreases binding (NE > E $\gg$ N-dimethyl-NE; isopropyl-NE: nil). In general only phenylethylamine derivatives with a β-hydroxyl group are found in vesicles, perhaps partly because amines entering without this group are β-hydroxylated in the vesicles. The β-hydroxyl group is important for binding (68) but does not appear to be essential since dopamine and α-methyltyramine can be found in heart microsomes,

TABLE 4

Binding of phenylethylamine derivatives in NE storage vesicles in vivo

Compounds in Estimated Order of Affinity for Binding Sites	Structure			References
	Ring-OH groups	β-OH group	Other groups	
α-Methyl epinephrine	2	Yes	α-Methyl	Well retained in vesicles[a]
NE	2	Yes		Well retained in vesicles (see text)
E	2	Yes	N-Methyl	Well retained in vesicles (51)
Dopamine	2	No		Moderate retention in microsomes especially when β-hydroxylase inhibited (46, 47)
α-Methyl-*m*-octopamine (Metaraminol)	1	Yes	α-Methyl	Probably in vesicles; retained in cardiac nerves (6)
m-Octopamine	1	Yes		Moderate retention in microsomes (47)
α-Methyl octopamine	1	Yes	α-Methyl	Moderate retention in microsomes (47)
Octopamine	1	Yes		Moderate retention in microsomes (46, 47, 64)
N,N-Dimethyl NE	2	Yes	N-Dimethyl	Slight retention in microsomes[a]
α-Methyl tyramine	1	No	α-Methyl	Measurable when β-hydroxylase inhibited (47)
Phenylethanolamine	0	Yes		Slight retention in microsomes[a]
Isopropylnorepinephrine	2	Yes	N-Isopropyl	Not retained in innervated tissues (32)
Dopa	2	No	—COOH	Not retained in innervated tissues[a]
Dihydroxymandelic acid	2	Yes	(Deaminated)	Not retained in microsomes[a]
Normetanephrine	1	Yes	O-Methyl	Not retained in microsomes (51)

[a] Potter, unpublished.

especially when the β-hydroxylase is inhibited by disulfiram. In addition, binding of dopamine (with 2 ring hydroxyl groups) exceeds that of octopamine and its derivatives (which have one ring and one β-hydroxyl group). The stereospecificity of the β-hydroxyl groups is very important, for binding of *l*-NE in the heart considerably exceeds that of *d*-NE given in low concentrations (36, 39, 42) and the binding of tracer amounts of *l*-NE in heart microsomes exceeds that of *dl*-NE by 40% (Potter, unpublished). Increasing the number of ring hydroxyl groups greatly enhances binding in vesicles (NE > octopamine ≫ phenylethanolamine). α-Methylated phenylethanolamine derivatives, particularly metaraminol, are well retained in nerves and vesicles (demonstrated for α-methyl-E and octopamine), probably because they are poor substrates for MAO rather than because of effects of the α-methyl group upon binding *per se*. O-Methylation abolishes binding in vesicles, although normetanephrine may be retained in nerve endings (63).

A distinction may be made between the compounds discussed here, which can compete with NE for binding in vesicles, and a group of compounds structurally similar to NE which interfere with uptake of NE (37) at a cellular level, with or without effects also on vesicles. With both groups of compounds phenolic hydroxyl groups and α-methylation increase, and N-substitution and O-methylation decrease affinity. However, β-hydroxylation increases binding of amines in vesicles whereas it has the opposite effect on the ability of a compound to compete with NE for uptake into nerves.

RELATION OF VESICLE-BOUND AND "SOLUBLE" NE TO "POOLS" OF THE TRANSMITTER

The fact that no more than 77% of the NE of the rat heart (51) or 35% of the NE in splenic nerve "press juice" (15) has been isolated in small particles, raises the question of the function and relation of the soluble NE to vesicle-bound NE. Prolonged homogenization permits better cellular disruption but also decreases the yield of amine-containing particles (38); repeated sedimentation and resuspension of particles has a similar effect. For this reason it has been suggested that much of the NE appearing in soluble form is released from storage vesicles during the process of cell disruption (51). On the other hand some NE must normally be present in the cytoplasm in equilibrium with that in the vesicles. Euler and Lishajko (18) have suggested that this soluble form is important as the source of the transmitter released by nerve stimulation. As yet the only information pertaining to this point is the demonstration of the quantal nature of postsynaptic potentials in the smooth muscle cells of the vas deferens (4) which may indicate release of NE from the hypogastric nerve in packets, corresponding to the amounts of NE in vesicles within the nerves.

Ample evidence has been presented that NE is present in more than one "pool" within tissues. "Compartments" of NE have been suggested on the basis of differential drug responses (7, 54, 66), different uptake rates (36), different release rates and modes of metabolism (40, 45) and different specific activities of NE present in the heart at different times (5, 51, 52, 53). These results may be

TABLE 5

In vitro studies of NE storage vesicles

Variable	NE Content in Splenic Nerve Particles (S) and Small Heart Microsomes (H)
Uptake of NE	S Restoration of depleted vesicles to original content in 6 to 12 × 10^{-5} M amine (18, 20) Exchange of labeled amine (6 × 10^{-6} to 10^{-4} M) with some of endogenous content (19) Partial exchange of content with 10^{-7} M amine; rapid at 37° C (65) Some stereospecificity of uptake for *l*-NE and *l*-E at 6 × 10^{-6} M (23) H Immediate adsorption of 17 times solution concentration (10^{-7} M) to particles at 4° C; further uptake to 36 times in 120 min at 23° C. Final content related to solution concentration even to 3 × 10^{-3} M. Uptake more inhibited by *l*- than *d*-isomer (52) Uptake: at 23° > at 37° > at 4° C and directly related to concentration of solution between 6 × 10^{-8} and 10^{-4} M; dopamine and 5-hydroxytryptamine, but not dopa or E concentrated in particles (29) Uptake observed in 10^{-6} to 10^{-4} M amine at 37° C. Metabolite also bound (44)
Reserpine	S Inhibits release or uptake between 10^{-8} and 10^{-5} M (16, 19, 22, 65) Causes release 7–70 × 10^{-5} M (13) H No inhibition of release when reserpine given *in vivo* (29), or *in vitro* at 10^{-6} to 10^{-3} M (52) "Uptake" inhibited only at 10^{-3} M; considerable release at 10^{-4} to 10^{-3} M (52)
Tyramine	S Releases at about 10^{-4} M (14, 65) H Releases and inhibits uptake 10^{-4} to 10^{-3} M (29, 52)
ATP	S Stimulates uptake and prevents spontaneous release (19, 21, 65) H Unclear stimulation of uptake (52)
Phenoxybenzamine	S Prevents release at 10^{-5} to 10^{-3} M (26, 65)
Cocaine	S Prevents release at 10^{-3} M (22) H Causes slight release at 10^{-3} M; no effect on uptake at 10^{-3} M (52)
Acetylcholine γ-Aminobutyric acid Histamine Guanethidine Bretylium 5-Hydroxytryptamine γ-Methyl *m*-tyrosine Xylocholine Nicotine β-Phenylisopropylhydrazine Ouabain Chlorpromazine	S, H No significant effects on uptake or release at or below 10^{-3} M (see 15, 22, 52)

the consequences of: 1) different types of binding of NE within a single population of vesicles (such as might occur between the quaternary N^+ group, any combination of one, two, or three hydroxyl groups, and the binding substance); 2) unequal distribution of vesicles within a tissue such as the heart or even within single nerves, leading to different availability of amines or drugs to binding sites; 3) the effect of different rates of release of NE from a single type of binding site (*e.g.*, by tyramine or reserpine) causing different availability of the released amines to intra- and extra-neuronal enzymes; and 4) the unquestionable presence of NE in both vesicular and axoplasmic "pools" (53, 56).

The available evidence indicates that vesicles represent the only *binding site* for NE within nerves. First, only one population of vesicles containing NE has been isolated. Attempts to demonstrate binding of the amine to soluble isolated proteins have not been successful (38). Second is the remarkable avidity of the vesicles for NE: within several minutes after the intravenous administration of H^3-NE to animals the ratio of microsomal NE to "soluble" NE reaches a constant value (65) which is maintained for 24 hr (38, 51). Third, the specific activities of NE in the soluble and vesicle fractions reach the same value within several minutes (43), a circumstance that suggests either that complete exchange of amines between the vesicles and axoplasm can occur within this time, or more likely, that most of the administered NE becomes bound in the vesicles and disruption of any number of them during isolation procedures leads to the same specific activity in the soluble and vesicle fractions thereafter. Finally, the same ratio of particulate NE to soluble NE is found before and after depletion of a tyramine-releasable pool of NE (38). It is not incompatible with this point that some tissues may show a low ratio of particle NE to soluble NE [*e.g.*, the uterus (72)] and that different ratios obtain for the presence of different phenylethylamine derivatives within nerves (47).

IN VITRO STUDIES OF NE STORAGE VESICLES

A large number of observations has been made *in vitro* of the interaction of isolated particles containing NE with drugs and with NE. An attempt has been made to collect these in table 5, but details should be sought in the references cited. Unfortunately, in many instances the conditions and drug concentrations used in these studies and the impurity of the preparations tested make it difficult to correlate these findings directly with physiological events.

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