Recent Views on Mechanisms for Lowering Sympathetic Tone

By Bernard B. Brodie, Ph.D.

All drugs that influence nerve endings were once believed to act directly on receptor sites, by mimicking or blocking the action of a neurohormone. In recent years many drugs have been shown to influence the nervous system indirectly by modifying the amount of a free, neurohormone at receptor sites. For example, reserpine changes the quantity of a free monoamine at receptors by impairing its sites of storage. Drugs also alter the quantity of a free monoamine by inhibiting its synthesis, metabolism, or physiologic release. It seems logical to consider all these sites of drug action as parts of an organized molecular unit responsible for the formation, storage, inactivation, and physiologic release of a neurohormone.

Since these molecular units transform one kind of energy into another, the term neurochemical transducer has been coined to describe them. Thus, electrical impulses arriving at the nerve ending act on the transducer and are translated into a quantity of free hormone. The free amine in turn acts on a target organ to produce chemical or mechanical energy, or on another neuron to produce additional electrical impulses (fig. 1). The neurochemical transducer contains a store of norepinephrine, epinephrine, dopamine, serotonin, or acetylcholine and is present in each of the billions of synapses and nerve endings in the body. They are really the primary units of behavior, since the organism responds to environmental change only because these units can regulate precisely the quantity of free neurohormone at nerve endings.

The functional organization of these units is one of the fundamental problems in biology. However, the efforts by the biochemists, physiologists, and pharmacologists to disclose the nature of these molecular units have been hampered by failure to study them as complete entities rather than considering them as separate processes of synthesis, storage, and metabolism. Homogenization destroys the structural integrity of these organized units and it is necessary to study them in the living animal or in the intact organ tissues.

The availability of tritium-labeled norepinephrine (H³NE) of high activity has permitted observations of the properties of sympathetic nerve endings in vivo from which a structure of the neurochemical transducer for the catecholamine can be formulated. Similar studies with labeled serotonin have been carried out but will not be reported in this paper. Although the concept is still schematized and fragmentary and, at best, only a tentative working hypothesis, it is proving a valuable framework on which to predict the action of drugs.

Concept of Organized Molecular Units at Sympathetic Nerve Endings

I shall describe briefly our speculations about the nature of the biophysical units at sympathetic nerve endings as well as the data on which these speculations are built. Since norepinephrine does not freely diffuse onto receptor sites and is relatively stable in tissues, it must be sequestered by an intracellular boundary, presumably lipoidal in nature (fig. 2). Since the concentration of norepinephrine at nerve endings is much greater than in extracellular fluid, something more than a lipoidal membrane is needed to explain its storage.

The nature of the storage mechanism has been explored by kinetic studies of the uptake of labeled monoamines by various tissues. The
results indicate that active transport is the key process which maintains the amines in various tissues.

For example, studies from this laboratory have shown that the high level of serotonin in blood platelets is maintained by a specialized transport system which is inhibited by reserpine, ouabain, and N-ethylmaleimide. When tracer amounts of radioactive serotonin are added to a platelet suspension, after a steady-state with unlabeled amine has been established, the uptake of labeled amine is still rapid although the net uptake of total amine is zero, indicating that the concentrating mechanism is acting continuously by a "pump and leak" mechanism.

The storage of norepinephrine in nervous tissue is a more complex process since sites of storage, formation, and inactivation are all together, and amine-containing granules are present. Kinetic studies by Titus et al. from this laboratory have shown that brain and heart slices take up H3NE against a concentration gradient by a process that is blocked by reserpine and poisoned by ouabain. The kinetics of uptake indicate that active transport is an integral part of the mechanism holding endogenous norepinephrine in storage and that this mechanism can also be regarded as a pump which resists the free outward diffusion of the amine formed inside the storage compartment. Included in these experiments was one showing that the preloading of brain slices with unlabeled norepinephrine does not slow the uptake of H3NE, indicating in another way that the uptake system is continually acting.

These investigators pointed out that isotopic equilibrium between labeled norepinephrine in medium and in slices is not reached by the time that the H3NE level within the slices has reached a plateau. They concluded from these results that active transport takes up the labeled norepinephrine into a readily available pool from which it mixes more slowly with a second or reserve pool.

The storage compartment illustrated in figure 2 shows an active transport mechanism (heavy arrow) acting on a mobile norepinephrine pool which is in equilibrium with a reserve pool held in granules, presumably a complex of norepinephrine with intragranular components. Since norepinephrine is formed inside the storage compartment, the active transport system can be pictured as a pump (heavy arrow) which resists the outward passive diffusion of the amine (dotted arrow). It is important to keep in mind that despite the low diffusion coefficient of norepinephrine across a lipoidal membrane, its rate of simple diffusion will increase with the increase in the norepinephrine concentration in the mobile pool until the force of outward

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**Figure 1**

A neurochemical transducer.

**Figure 2**

Schematic diagram showing a proposed arrangement of the neurochemical transducer. It consists of a lipoid membrane with an active transport system or pump (heavy arrow) to control passage of norepinephrine from storage compartment to receptor and metabolic sites. The light arrow represents leakage of norepinephrine by passive diffusion. MAO in mitochondria prevents norepinephrine from leaking onto receptor. The physiological release of amines from mobile pool is regulated by nerve impulses which change membrane permeability. The amines in granules may be a reserve pool in equilibrium with the mobile pool.
diffusion will finally counteract the inward force of active uptake. This combination of an active transport system and passive diffusion is commonly called a "pump and leak" system.

The model must explain how the norepinephrine content is maintained constant, without the amine overspilling onto receptors. On this point there has been disagreement; some believe that the content of norepinephrine is controlled by a feedback mechanism which stimulates its rate of synthesis as the stores are expended; others think that the catecholamine content is kept constant by continuous synthesis and wastage. The second view seemed more plausible to us, for it is a general rule in nature that stored substrates undergo continuous turnover, whether they are being utilized or not.

Recently we have established that norepinephrine in peripheral nerve endings is formed continuously even in the absence of sympathetic tone. For these studies we have used H3NE to measure the rate of norepinephrine biosynthesis. In the past the formation of a catecholamine has been measured from the rates of disappearance or formation of the labeled substance formed from a radioactive precursor. It is not generally appreciated, however, that norepinephrine in sympathetic nerve endings can be labeled directly, by taking advantage of the active transport system which will take up H3NE from the blood stream into nerve endings.

As first reported by Axelrod and his associates, H3NE is taken up by various tissues. They concluded that the labeled amine is absorbed by sympathetic nerve endings since it does not accumulate in tissues after sympathetic denervation. In our studies tracer amounts of highly labeled H3NE were injected intravenously into rats, guinea pigs, and mice. A few minutes later the H3NE level in heart was 50 or more times higher than that in blood, suggesting that the labeled amine was taken up by the same active transport system which maintains endogenous norepinephrine inside nerve endings. Our results in figure 3, which describe the kinetics of the decline in radioactivity, indicate that H3NE is first taken up into a readily miscible pool from which it slowly diffuses into a second pool. The initial decline in the heart H3NE is relatively rapid but becomes exponential within 12 hours in the rat and guinea pig, and within 4 hours in the mouse. The exponential decline suggests that H3NE has equilibrated throughout all the stored epinephrine and that the decline in radioactivity results from the continuous production of new unlabeled norepinephrine and the simultaneous removal of norepinephrine, labeled and unlabeled. Since the quantity of endogenous norepinephrine remains constant, the slope of the decline represents the combined turnover of both norepinephrine pools. For reasons described below, this turnover may be less than the actual rate of norepinephrine formation.

The diphasic decline in specific activity suggests that endogenous norepinephrine is stored in an open two-compartment system, as

![Figure 3](image-url)

*Figure 3*

**Time-course of H3NE content in heart after i.v. administration of labeled amine to rats.**
shown in figure 4. Immediately after the injection of \( H^3 \)NE, the radioactivity is confined to the mobile pool \( S_1 \) into which newly formed norepinephrine readily enters and from which the endogenous amine diffuses or is released by nerve impulses. Since most of the trafficking in the nerve ending goes on in this pool, it follows that during the time that it contains most of the \( H^3 \)NE, the decline in radioactivity will be most rapid. As the \( H^3 \)NE exchanges with \( S_2 \), the reserve pool, the decline in radioactivity will become less abrupt and at equilibration will be exponential. Conventionally, \( S_1 \) may be regarded as the precursor and \( S_2 \) the product—perhaps a complex of norepinephrine with ATP and other cellular components—and the specific activity of each pool will have the classical precursor-product relationship: the specific activity of \( S_2 \) starting from zero will progressively increase until it exceeds that of \( S_1 \) and declines in parallel. The ratio \( S_{A2}/S_{A1} \) at equilibration will depend largely on the rate of norepinephrine exchange between the pools. If this rate is high, the specific activities will be almost equal and the rate of norepinephrine formation will not differ much from the turnover rate calculated from the exponential decline in \( H^3 \)NE. If the rate of exchange is slow, the specific activity of \( S_2 \) will be higher than of \( S_1 \) and the actual rate of norepinephrine biosynthesis will be much greater than the turnover rate calculated from the decline of \( H^3 \)NE. In any case, a change in norepinephrine formation will be reflected by a corresponding change in the slope of the \( H^3 \)NE decline.

Figure 3 is the sum of two exponentials, whose slopes can be readily calculated. From these values can be calculated the various fractional turnover rates, the relative pool sizes, \( S_1 \) and \( S_2 \), the rate of norepinephrine synthesis and the ratio of specific activities, \( S_{A2}/S_{A1} \), at equilibrium (fig. 4). The latter will indicate how much the actual rate of norepinephrine synthesis differs from that calculated from the decline in \( H^3 \)NE. Preliminary estimates suggest that in rats, mice, and guinea pigs, \( S_1 \) is about 40 per cent of the total norepinephrine, that \( S_{A2}/S_{A1} \) at equilibration is about 2.5, and that the rate of norepinephrine synthesis is about twice that calculated from the decline in \( H^3 \)NE. The rate of synthesis is roughly 0.12 \( \mu \)g./Gm./hr. for the mouse, 0.17 \( \mu \)g./Gm./hr. for the guinea pig and 0.06 \( \mu \)g./Gm./hr. for the rat.

A note of precaution is given before these results are taken at face value. We used \( dl \)-norepinephrine-\( H^3 \) and have made the assumption that the nerve endings selectively take up the \( l \)-isomer. This is probably true if \( H^3 \)NE is given in truly tracer amounts. However, for a short time after the rapid injection of relatively large amounts of \( H^3 \)NE (about 3 to 6 \( \mu \)g./Kg.) the level of \( d \)-isomer in the extracellular fluid is so high that appreciable amounts might be taken up by passive diffusion. To the extent that the \( d \)-isomer is taken up and disappears at a different rate from the \( l \)-isomer, our results will require quantitative though not qualitative amendment.

Since the formulation of the neurochemical transducer is schematic, a number of anatomic models could satisfy the kinetics of the two-compartment system of figure 4. For example,
the membrane of each granule in the nerve ending might possess a specialized transport system which maintains a mobile norepinephrine pool in equilibrium with a "chemically bound" pool. Recent studies by others\textsuperscript{12,13} have shown that granules from the adrenal medulla take up labeled epinephrine in the presence of adenosine triphosphate and Mg\textsuperscript{2+} ions in vitro by a process which is blocked by reserpine and various metabolic inhibitors and has the kinetics characteristic of active transport. When the level of the label within the granules reaches a plateau, it is mixed with only a fraction of the endogenous norepinephrine, suggesting that the granules contain two pools of the amine.

The origin of the norepinephrine-containing granules is a puzzling problem since it is unlikely that they are formed in the nerve endings. It is possible that they are made in the cell bodies. Recent work, utilizing a new histologic technic for the fluorescent staining of norepinephrine in tissue slices, indicates the presence of significant amounts of the amine in the cell bodies of sympathetic ganglia.\textsuperscript{14} In addition, norepinephrine granules are found in the axons of postganglionic sympathetic nerves.\textsuperscript{15} These results suggest that the granules are formed in the cell bodies and carried down to the nerve endings through channels in the endoplasmic reticulum.

With reference again to figure 2, the exponential decline of H\textsuperscript{3}NE over a period of 72 hours suggests that the rate of norepinephrine formation is constant, irrespective of nerve stimulation. We have obtained experimental proof for this view in experiments in which sympathetic tone is abolished for 24 hours by means of mecamylamine; the turnover of norepinephrine remains virtually unchanged in the absence of sympathetic tone.

The demonstration that the synthesis of norepinephrine is uninterrupted is counter to the view that synthesis is controlled by nerve stimulation; like other substrates stored in the body, norepinephrine is constantly formed and inactivated irrespective of its utilization. A constant turnover ensures the availability of the amine in amounts far in excess of the immediate need.

With continuous biosynthesis of norepinephrine, a regulatory mechanism is needed to ensure that the amine does not overrun onto the receptors. Here we conceive the true role for monoamine oxidase—preventing the uncontrolled release of norepinephrine onto receptor sites.\textsuperscript{16} This function is best described on kinetic grounds. MAO in mitochondria, placed contiguous to the storage compartment (fig. 2), will inactivate small amounts of norepinephrine, which constantly diffuse across the membrane. As a result, the outward diffusion of norepinephrine is facilitated and the steady-state level of norepinephrine is maintained well below that which will saturate the transport system.

Such a role for MAO stresses how unproductive are debates on the relative importance of MAO or catechol-O-methyl transferase in the metabolism of catecholamines. In biology this question is best rephrased to—what is the role of each enzyme in the intact animal? MAO is important in regulating the amount of stored norepinephrine; catechol-O-methyl transferase is certainly the important enzyme for inactivating circulating catecholamines, though the physiologic significance of this function is uncertain.

The model of the neurochemical transducer must also depict how nerve impulses release norepinephrine onto the receptors. After electrical stimulation of sympathetic nerves labeled with H\textsuperscript{3}NE, the radioactivity leaving the nerve endings consists mainly of the bases, norepinephrine and normetanephrine.\textsuperscript{17} Hence, the nerve impulse may be assumed to release norepinephrine by countering the pump in that part of the membrane facing the receptor (fig. 2); the nerve impulse may block the pump vis-a-vis the receptor site or make the membrane so porous that the pump becomes ineffectual.

Between successive nerve impulses the action of the pump is restored. Since the receptor and the storage membrane are extremely
close, the pump will suck the norepinephrine on the receptor back into the nerve ending. Brown has concluded that even after a train of nerve impulses, much of the norepinephrine released onto the receptor is returned to storage.  

Thus, the neurochemical transducer described by figure 2 can explain why the norepinephrine content of nerve endings remains constant, even after repeated stimulation. The thrifty use of norepinephrine at the receptors together with a persistent synthesis ensures that the nerve endings are never depleted under ordinary circumstances. Thus, the small quantity of norepinephrine in the nerve endings acts as though it were an infinite supply. An additional factor which might regulate norepinephrine stores is chemical in nature. If the concentration of norepinephrine in the mobile pool is high enough to exert an inhibitory effect on dopamine \( \beta \)-oxidase by mass action, the rate of norepinephrine synthesis would automatically increase with a decrease in the concentration of stored norepinephrine. This factor might explain the rapid formation of heart norepinephrine after depletion by a compound whose action is short-lasting. Moreover, it is pertinent that dopamine exerts an inhibitory effect on DOPA decarboxylase in vitro.

The neurochemical transducer concept has proved valuable in studies of physiologic problems involving the sympathetic system. One such problem is the relationship between thyroid action and the sympathetic system. Since certain features of the hyperthyroid state are reminiscent of those of a high sympathetic tone including excitability, increased metabolic rate, disturbance of fat and carbohydrate metabolism, tachycardia and high pulse pressure, we entertained the possibility that these signs are associated with an increased rate of norepinephrine synthesis.

To test this view mice were made hyperthyroid. \( H^3NE \) was then administered and its rate of disappearance measured as described previously. The results in figure 5 show that the turnover rate of heart norepinephrine is the same in normal and hyperthyroid animals. The somewhat lower counts per gram of heart tissue in the hyperthyroid animals is accounted for mainly by the increased size of this organ.

Since the thyroid hormone is known to potentiate the effects of administered catecholamines, the high sympathetic tone in hyperthyroidism may be attributed to sensitization of sympathetic receptors. In fact, Maickel and co-workers in our laboratory have shown that the lipolytic response of adipose tissue to norepinephrine is considerably greater in epididymal fat pads taken from hyperthyroid rats than from normal animals and that fat pads from thyroidectomized rats show little lipolytic response. Preliminary results suggest that the thyroid hormone increases the amount of lipase in adipose tissue.

**Figure 5**

Disappearance of \( H^3NE \) in heart of hyperthyroid and control mice after administration of \( H^3NE \). Open circles: control mice. Closed circles: hyperthyroid mice.
Table 1
Effects of Drugs on the Norepinephrine Neurochemical Transducer

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<tbody>
<tr>
<td>1.</td>
<td>Mimics action of norepinephrine at reactive sites</td>
</tr>
<tr>
<td>2.</td>
<td>Mimics action of norepinephrine by releasing small amounts of the amine onto reactive sites</td>
</tr>
<tr>
<td>3.</td>
<td>Blocks action of norepinephrine at reactive sites</td>
</tr>
<tr>
<td>4.</td>
<td>Blocks metabolism of norepinephrine at the storage compartment</td>
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<tr>
<td>5.</td>
<td>Blocks norepinephrine storage processes</td>
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<tr>
<td>6.</td>
<td>Blocks norepinephrine synthesis</td>
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<tr>
<td>7.</td>
<td>Blocks physiologic release of norepinephrine</td>
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<tr>
<td>8.</td>
<td>Activates physiologic release of norepinephrine</td>
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The Action of Drugs on the Neurochemical Transducer

A crucial test of the neurochemical transducer concept is its value in explaining the action of drugs. According to the model presented in figure 2, drugs should affect blood pressure by acting on the neurochemical transducer in one of eight different ways (table 1).

1. Compounds (norsynephrine and other norepinephrine analogues) mimic the action of norepinephrine at peripheral nerve endings.

2. Many drugs (mainly phenylethylamine analogues) mimic the action of norepinephrine by releasing a small amount of the amine. Thus, amphetamine does not exert a sympathomimetic effect after animals are depleted of norepinephrine by reserpine. The action of amphetamine is not completely clear, however, since it still acts on central adrenergic centers in animals pretreated with reserpine.

3. Dibenamine and other adrenergic blocking agents prevent the action of catecholamines by occupying peripheral receptor sites. Chlorpromazine is said to have a similar action in the brain.

4. After the blockade of brain MAO, the norepinephrine stores are increased in brain, sympathetic ganglia, and heart and the free amine diffuses from the nerve endings into surrounding spaces (fig. 6). In brain the blood-brain barrier restrains the poorly lipidsoluble amine from readily diffusing into the blood stream and it spills over onto receptor sites. The question has been raised whether the stimulatory effects produced by MAO inhibitors are caused by free serotonin or by free norepinephrine. Present evidence indicates that neither serotonin nor dopamine is responsible and that there is an excellent correlation between the rise in free norepinephrine and central stimulation. The effect of MAO inhibitors on blood pressure is another story and is discussed at the end of this paper.

The scheme in figure 6 also explains why reserpin e given shortly after a MAO-blocking agent does not cause a rapid decline in brain norepinephrine. Although reserpine releases norepinephrine from the restraint of the "pump," the free amine is no longer inactivated by MAO. Since norepinephrine is literally trapped by the blood-brain barrier, the amine becomes available to reactive sites. The conclusion that MAO inhibitors counteract the reserpine-induced depletion of norepinephrine is contradicted by the pronounced excitation observed in rabbits.

5. The main steps in the biosynthesis of catecholamines are as follows:

Tyrosine → DOPA → Dopamine → Norepinephrine. The synthesis of norepinephrine would be prevented by blockade of either DOPA decarboxylase or dopamine-β-oxidase. Attempts to block DOPA decarboxylase suffer the complication that DOPA and 5-hydroxytryptamine are decarboxylated by the same en-
zyme,\textsuperscript{27} hence a substance which blocks the formation of dopamine will also block that of serotonin and other aromatic amines. A further difficulty is the large excess of the enzyme in nervous tissue together with the competitive nature of the inhibitors. Our studies have taught us not to conclude that the synthesis of an amine is prevented in vivo on the basis of the blockade of enzyme activity measured in vitro. This lesson arose from the study of two classes of compounds (see below), which are particularly potent as competitive inhibitors of DOPA decarboxylase\textsuperscript{28, 29}

\begin{center}
\begin{tikzpicture}
\node [draw] (n1) at (0,0) \text{NSD 1024};
\node [draw] (n2) at (1,-1) \text{NSD 1034};
\node [draw] (n3) at (0,-2) \text{HO} ;
\node [draw] (n4) at (1,-3) \text{HO} ;
\node [draw] (n5) at (-0.5,-1) \text{CH}_{3} ;
\node [draw] (n6) at (-0.5,-2) \text{-CH}_{2} \text{ONH}_{2} ;
\node [draw] (n7) at (0.5,-1) \text{-CH}_{2} \text{N-NH}_{2} ;
\end{tikzpicture}
\end{center}

The administration to mice of NSD 1034 (100 mg./Kg.) inhibits the activity of brain DOPA decarboxylase by 100 per cent as measured by the assay of the enzyme activity in brains removed from these animals. In the intact animals, however, the formation of endogenous dopamine and serotonin is barely affected and the brain levels of these amines still rise after blockade of MAO.\textsuperscript{30} This apparent paradox is a good illustration of the difficulty of extrapolating the inhibitory effects of drugs in vitro to functional inhibition in vivo. In the living animal, the enzyme or substrate might be localized in large concentrations at specific sites in nerve endings and be greatly diluted in tissue homogenates. A competitive inhibitor might be potent enough to block the formation of dopamine from DOPA in the homogenate, but not in the living animal.

In passing, it must be pointed out that \textalpha-methyl-dopa is a much less potent inhibitor of DOPA decarboxylase than NSD 1034, and that the decline in norepinephrine produced by the former compound does not result from decarboxylase inhibition but from release of the amine.\textsuperscript{30, 31}

Blockade of dopamine-\beta-oxidase is a more practical way of blocking norepinephrine synthesis, since the hydroxylation of dopamine is a rate-limiting step. We discovered rather fortuitously that a number of the decarboxylase inhibitors mentioned above are extremely potent inhibitors of dopamine-\beta-oxidase.\textsuperscript{32, 33} In mice, they prevent the rapid rise in brain norepinephrine level induced by a potent MAO inhibitor (pargyline).\textsuperscript{32} Moreover, after brain norepinephrine and serotonin have been released by a short-acting benzoquinolizine derivative, these inhibitors prevent the rise in brain norepinephrine but not the rise in serotonin.

The pharmacologic consequences of a complete blockade of norepinephrine biosynthesis is unknown. The norepinephrine level might not decline rapidly; this will depend on the utilization rate of the stored amine which, in turn, will depend on nervous activity. Unfortunately, presently available inhibitors are short-lasting and do not appreciably lower stores of endogenous norepinephrine. We hope that more potent and longer-lasting inhibitors will soon be available.

6. Reserpine and certain other Rauwolfia alkaloids impair the transport system which maintains monoamines in their mobile pools.\textsuperscript{16} As a result, the amines diffuse through the storage membranes and are metabolized by MAO (fig. 7). Since the mobile and reserve pools are in a state of equilibrium, the intra-granular amines will also reach MAO by way
of the mobile pool. Although reserpine releases considerable amounts of norepinephrine, sympathetic activity is not markedly enhanced since the amine is released mainly onto MAO and the radioactivity from hearts previously labeled with H3NE appears in the blood almost entirely as acidic metabolites. In contrast, after a train of nerve impulses the radioactivity appears mainly as the bases (norepinephrine and its methylated derivative). Similarly, Kopin et al. have shown that reserpine increases the urinary excretion of radioactivity, much of which is in the form of the deaminated products.

The problem often arises whether reserpine affects two different mechanisms in releasing and blocking the uptake of norepinephrine. Different mechanisms for the uptake and release seem an unnecessary complication for if the pump part of a "pump and leak" process is inhibited, the loss of the catecholamines is explained by unopposed diffusion.

After reserpine inhibits norepinephrine storage, the amine is still formed at peripheral nerve endings but at such a slow rate that there is a deficiency of free norepinephrine at receptors. In contrast, norepinephrine in brain is formed relatively rapidly and after reserpine administration, the level of free amine at synapses is high enough to elicit a normal or even an increased sympathetic output from the central nervous system. It may be concluded that the lowering of blood pressure by reserpine is not a central but a peripheral effect, owing to the loss of norepinephrine at peripheral nerve endings.

It is not generally appreciated that reserpine given in very small doses can deplete much of the peripheral norepinephrine without releasing brain amines; hence in low doses the drug may lower blood pressure without producing appreciable central effects. A more complete separation of the central and peripheral effects is obtained with syrosingopine, a semisynthetic drug, structurally related to reserpine. In large doses, however, this compound also produces sedation. A reserpine analogue which would release only peripheral norepinephrine and not brain amines would have obvious advantages in the treatment of hypertension.

7. Bretylium, a quaternary ammonium compound, acts as a sympatholytic agent by interfering with the physiologic release of norepinephrine at peripheral sympathetic nerve endings (fig. 8). BW 392C60, a strongly basic guanidine derivative, also has a side-chain nitrogen separated from a ring by a single carbon atom:

![Schematic diagram showing the effects of blocking the physiological release of norepinephrine by bretylium and BW 392C60.](image)
This drug acts like bretylium but is far more potent.  

8. Guanethidine, a guanidine derivative, produces a variety of sympatholytic effects including the lowering of arterial pressure. The drug releases norepinephrine from peripheral sympathetic neurons but not from the brain (fig. 9), since the drug does not cross the blood-brain barrier in appreciable amounts. We are entertaining the possibility that guanethidine acts oppositely to bretylium, and depletes peripheral stores of norepinephrine by persistent activation of the mechanism by which nerve impulses trigger the release of the amine. In accord with this view, pretreatment of rats with bretylium prevents guanethidine, but not reserpine, from lowering the heart catecholamine level, a result which indicates that reserpine and guanethidine liberate the amines by different mechanisms (table 2). In addition, guanethidine produces a sympathomimetic effect lasting 90 minutes in the spinal cat; neither bretylium nor BW 392C60 exerts this action (fig. 10). That guanethidine and reserpine deplete norepinephrine by different mechanisms is also shown by the different ways in which they release the amine. Reserpine blocks the norepinephrine pump, thereby allowing the amine to diffuse onto MAO and form deaminated products. In contrast guanethidine releases the norepinephrine directly onto the receptor sites; indeed recent studies from our laboratory show that guanethidine releases H<sup>3</sup>NE from the heart largely in the form of the bases, norepinephrine and normetanephrine. In other words, guanethidine does not affect the norepinephrine pump but seems to release the catecholamines directly onto the receptors.

Reports in the literature suggest that guanethidine acts primarily by the same mechanism postulated for bretylium, with the depletion of norepinephrine a secondary effect. We have shown, however, that a considerable number of analogues of BW 392C60 exert a bretylium-like effect and also prevent the release of norepinephrine by guanethidine. Consequently, if guanethidine also has a bretylium-like action it is difficult to understand why it should not prevent its own release of norepinephrine. The statement that guanethidine acts like bretylium is based on the finding that shortly after its administration the sympathetic nervous system fails.
to respond to an electrical stimulus, although the norepinephrine stores are not yet depleted. Perhaps the nerve ending cannot release norepinephrine any more rapidly than it is already being released by guanethidine.

Guanethidine might first release norepinephrine from the mobile pool after which the release of the amine would be limited by the rate at which the amine leaves the granular reserve pool. The pattern of release by guanethidine is in accord with this view. After the first hour the release of norepinephrine seems to follow a zero order reaction as though the rate-limiting step is the rate at which the reserve pool is liberated.

Preliminary studies suggest that α-methyl-metatyrosine and α-methyldopa might act similarly to guanethidine. For example, metaraminol (m-hydroxynorephedrine), a decarboxylation product of α-methyl-metatyrosine (α-MMT) is an extraordinarily potent depleter of peripheral norepinephrine. Our studies show that after metaraminol is given, the radioactivity from the heart labeled with H3NE appears almost entirely as bases (norepinephrine and normetanephrine), suggesting that metaraminol, like guanethidine, releases norepinephrine onto receptor sites. Since α-methyl-metatyrosine and α-methyldopa lower the norepinephrine levels through the action of their products of decarboxylation, it may be presumed that these amino acids also release norepinephrine directly onto receptors.

Since amphetamine also appears to release norepinephrine onto receptors, it is therefore of considerable interest that small doses of amphetamine, metamphetamine, and ephedrine not only prevent guanethidine from depleting norepinephrine but counteract the pharmacologic effects of guanethidine.

**Hypotensive Effects of MAO Inhibitors**

Clinically used MAO inhibitors generally lower blood pressure. Our concept of the neurochemical transducer has helped us in studies of the mechanism of this action. After Dr. Gessa demonstrated that the potent bretylium-like compound, BW 392C60, is a potent MAO inhibitor in vitro, though not particularly potent in vivo, we investigated whether other MAO inhibitors elicit a bretylium-like effect. Table 3 shows that a number of these drugs prevent the decline in heart norepinephrine produced by guanethidine, some of them being more active than bretylium. Since bretylium and BW 392C60 also prevent the nerve impulse-induced release of norepinephrine, the possibility was entertained that these compounds and MAO inhibitors might lower blood pressure by similar mechanisms.

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<tr>
<th>Drug</th>
<th>Relative antiganethidine activity</th>
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<tr>
<td>Bretylium</td>
<td>1.00</td>
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<tr>
<td>BW 392C60</td>
<td>7.00</td>
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<tr>
<td>Harmaline</td>
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<td>Tranylepromine</td>
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</tbody>
</table>

Rats were pretreated with one of the drugs, 2 hours later given guanethidine (10 mg./Kg. i.p.) and then killed 5 hours later. The dose of each drug was the minimal dose that inhibited depletion of norepinephrine by 90 per cent. Heart norepinephrine content of controls was 1.1 µg./Gm.
MAO. With phenelzine and tranylcypromine, however, the two actions could not be separated.

We determined the effect of the drugs on the reactivity of the peripheral adrenergic nervous system to electrical stimulation of the celiac ganglion (fig. 11). Bretylium blocks the increase in blood pressure elicited by low frequency stimulation though not that elicited by high frequency stimulation; whereas the more potent drug BW 392C60 blocks the response even at high frequency stimulation. The MAO inhibitor, pargyline, like bretylium, blocks the response to low frequency stimulation but does not block the response to higher frequency stimulation. Phenelzine produces similar results. These antagonistic effects last for a period of at least 5 hours.

In other experiments we determined the effects of the drugs on the pressor response rise induced by the action of 1,1-dimethyl-4-phenylpiperazinum (DMPP) on peripheral sympathetic ganglia (fig. 12). BW 392C60 and bretylium not only block the rise in arterial pressure but reverse it. Pargyline and phenelzine also reverse the pressor effects of DMPP for a period lasting at least 5 hours but less than 15 hours.47

Several investigators have speculated that MAO inhibitors elicit orthostatic hypotension by interfering with transmission in sympathetic ganglia.48-50 Since MAO inhibitors do elicit some action on ganglia, experiments were carried out to establish the extent to which this action could prevent nerve impulses from reaching sympathetic nerve endings. In these studies synaptic transmission was compared before and after administration of pargyline. The MAO inhibitor was given for 3 days as in the previous experiments and changes in synaptic transmission were measured by applying electrical stimuli to the superior cervical sympathetic nerve and recording the evoked potential postganglionically. The results show that blockade of MAO is only partial and lasts 1 or 2 hours at most.47

These results indicate that MAO inhibitors of widely diverse structures exert a bretylium-like action; they counteract the decline in heart norepinephrine produced by guanethidine and prevent sympathetic nerve impulses from affecting nerve endings. Since the sympatholytic action of the MAO inhibitors is not related to blocking the action of catecholamine on receptor sites or to interfering with transmission in sympathetic ganglia, they presumably prevent nerve impulses from releasing norepinephrine onto reactive sites.

Several studies have shown, however, that MAO inhibitors do not relax the nictitating membrane nor block the contraction elicited by electrical stimulation of cervical sympathetic neurons. An explanation for this selectivity of action is essential in understanding the mechanism by which MAO inhibitors lower blood pressure. It is possible that the free norepinephrine spared by blockade of MAO counteracts the bretylium-like action. According to this view, the net effect of MAO inhibitors will depend on the amount of norepinephrine released spontaneously onto the

<table>
<thead>
<tr>
<th>Drug dose mg./Kg.</th>
<th>Percent MAO inhibition</th>
<th>Percent blockade of norepinephrine depletion</th>
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</thead>
<tbody>
<tr>
<td>Bretylium</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>BW 392C60</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>3</td>
</tr>
<tr>
<td></td>
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<td>45</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>53</td>
</tr>
<tr>
<td>Pargyline</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>25.0</td>
<td>53</td>
</tr>
</tbody>
</table>

Rats were first pretreated with one of the drugs, 2 hours later with guanethidine (10 mg./Kg. i.p.), and then killed 5 hours later. Guanethidine in control animals lowered heart norepinephrine level from 1.1 µg./Gm. to 0.1 µg./Gm.
Blood pressure responses to electrical stimulation of sympathetic ganglia. Adrenalec-
tomized cats were anesthetized with chloralose and rise in blood pressure recorded upon
electrical stimulation of the distal end of splanchnic nerve. Bretylium, BW 392C60, and
phenelzine were injected in single doses (as indicated in the figure) and response
to splanchnic stimulation again tested. In the case of pargyline the drug (50 mg./Kg.)
was given each day for 3 days preceding the experiment and 15 hours after the last
dose the blood pressure response to splanchnic stimulation was tested. Pargyline was
then infused (50 mg./Kg. i.v. during 10 minutes) and the response to splanchnic stim-
ulation was tested repeatedly during 5 hours.

Figure 11
reactive sites; the more rapid the turnover of norepinephrine the more free norepinephrine will be available to overcome the bretylium-like effect. It would be of great interest to compare the rates of norepinephrine biosynthesis in the nictitating membrane and in the blood vessels.

It is pertinent that the bretylium-like action elicited by MAO inhibitors is independent of their action on the enzyme. If anything, as pointed out above, MAO blockade should counteract the sympatholytic effects by causing an uncontrolled release of norepinephrine onto receptor sites. Perhaps the

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blockade of MAO might be regarded as an undesirable side effect in the use of these drugs as hypotensive agents. A better sympatholytic agent might be found in those MAO inhibitors which have been discarded because they are poor enzyme inhibitors.

**Summary**

A picture is gradually unfolding of the molecular structures at nerve endings which synthesize and store norepinephrine and release the amine onto sympathetic receptors. Norepinephrine is formed continuously regardless of sympathetic tone and is stored inside a lipid membrane. The amine is present in two pools—a reserve pool in granules in equilibrium with a mobile pool which is maintained against a concentration gradient by active transport. MAO controls the amount of norepinephrine in the nerve ending so that at the steady-state level the amine does not freely diffuse onto receptor sites. In the absence of sympathetic tone, norepinephrine can leave the storage compartments by simple diffusion through the lipid membrane onto MAO. After nerve stimulation the amine is released directly onto the receptor sites and reaches the blood stream in the form of bases.

This model for the transducer helps to explain the action of a number of drugs. Amphetamine increases blood pressure by releasing a small amount of norepinephrine at peripheral sympathetic nerve endings. Reserpine lowers blood pressure by inhibiting the norepinephrine pump which maintains the amine in the mobile pool; as a result the peripheral stores of amine are depleted by an uncompensated diffusion of the amine onto MAO.

Bretylium and a number of benzyl derivatives of guanidine lower blood pressure by preventing nerve impulses from releasing norepinephrine at sympathetic nerve endings. Guanethidine and a number of phenylethyl guanidine derivatives cause lowering of arterial pressure by depleting the peripheral stores of norepinephrine perhaps through a persistent activation of the physiologic release mechanism. Alpha-methyldopa, α-methylmetatyrosine, and metaraminol may act in a similar way.

Finally, considerable evidence suggests that MAO inhibitors lower blood pressure by exerting a bretylium-like action. They not only prevent guanethidine from releasing norepinephrine but like bretylium they appear to prevent nerve impulses from releasing norepinephrine onto receptors.

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