FALSE ADRENERGIC TRANSMITTERS[1]

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Introduction

The concept that excitation might be transferred from a nerve ending to an effector organ by the release of a chemical substance was developed over a century ago (1). Because of the similarity in the physiological effects of epinephrine and sympathetic nerve stimulation, Elliott, in 1905 (2), made the brilliant suggestion that an epinephrine-like compound was the substance released at sympathetic nerve endings. Nearly half a century passed before it was established that the epinephrine-like substance was norepinephrine. The evidence has been comprehensively reviewed by Von Euler (3).

During the last decade, the identification of the various metabolic products of the catecholamines and the discovery of drugs which alter their formation, storage, release, metabolism, transport, and physiological activity have resulted in a rapid advance in the knowledge of the physiology, biochemistry, and pharmacology of the adrenergic nervous system. These advances have been the subject of several symposia (e.g. 4–7) and numerous reviews.

Since the processes for synthesis, storage, and release of norepinephrine are not entirely specific, structurally related substances also may be formed and stored in the sympathetic nerve endings and released by nerve stimulation. Such "false neurochemical transmitters" vary in efficiency of interaction with the norepinephrine receptor and are generally less active than the physiological transmitter.

It is the purpose of this presentation to indicate the criteria which must be satisfied to establish that a substance is an "adrenergic false transmitter"; to review the evidence that a variety of phenylethylamine derivatives which meet these criteria can be synthesized and serve as false adrenergic transmitters *in vivo*; to examine the functional consequences of the accumulation of such substances; and to summarize how the concept of false transmitters has been used to further our knowledge of the physiology, biochemistry, and pharmacology of the sympathetic nerve ending.

Criteria for Adrenergic False Transmitters

Three major criteria are generally considered necessary to identify a compound as a neurotransmitter: the substance must be present in nerve endings in the same site as the physiological transmitter; it must be released by

[1] The survey of literature pertaining to this review was concluded in June 1967.

physiological nerve stimulation; and it must have the same physiological actions and pharmacological properties, in appropriate concentrations, as the natural transmitter. By analogy, criteria for a false transmitter may be similarly determined. Although not normally present in significant quantity in the nerve ending, a false transmitter can be made to accumulate in the same sites in the nerve endings as the physiological transmitter. The resulting accumulation may follow administration of the substance itself, a precursor or a drug which allows an endogenous compound to form the false transmitter. The false transmitter must be, of course, held in the same storage sites as norepinephrine, released by nerve stimulation, and depleted by drugs which deplete the physiological transmitter. There is no requirement, however, that the released substance be as active as the physiological transmitter; indeed, the activity of most adrenergic false transmitters is far less than that of norepinephrine.

Nonspecificity of Synthesis, Storage, and Release of the Adrenergic Transmitter

Synthetic enzymes.—Norepinephrine is formed from tyrosine by the sequential action of three enzymes. Tyrosine hydroxylase, which is responsible for the formation of 3,4-dihydroxyphenylalanine (DOPA) from tyrosine, is considered to be the rate-limiting enzyme in norepinephrine synthesis (8, 9). Decarboxylation to form DOPAmine is the next step in the sequence and is catalyzed by DOPA decarboxylase (10). In the final reaction DOPAmine-β-hydroxylase converts DOPAmine to norepinephrine (11).

The first of the three enzymes to be discovered was DOPA decarboxylase (10, 12). In their work with this decarboxylase, which was partially purified from guinea pig kidney, Lovenberg et al. concluded that a wide range of aromatic L-amino acids are its substrates (13). Several α-methylamino acids such as α-methyl DOPA and α-methylmetatyrosine are competitive inhibitors as well as substrates of this enzyme. DOPA decarboxylase isolated from other sources (adrenal medulla of the ox and human pheochromocytoma or argentaffinoma) was found to be more specific since *p*-tyrosine, histidine, and tryptophan were not substrates (14).

Although conversion of DOPAmine to norepinephrine had been demonstrated in adrenal medullary slices and in homogenates *in vitro* (15, 16), identification of cofactors and study of the specificity of DOPAmine-β-hydroxylase awaited solubilization and purification of the enzyme (17, 18). A wide variety of phenylethylamine derivatives are substrates for DOPAmine-β-hydroxylase (19–21). Thus phenylethylamine derivatives, which are formed by decarboxylation of some of the aromatic amino acids, can be converted to the corresponding phenylethanolamines. Decarboxylation of L-α-methylamino acids produces (+)-α-methylamines, the optical isomers of which are good substrates for DOPAmine-β-hydroxylase (21).

The relative nonspecificity of DOPA decarboxylase and DOPAmine-β-hydroxylase observed *in vitro* are relevant *in vivo*. A variety of phenolic acids

are excreted in human urine (22) and appear to be metabolic products of the action of monoamine oxidase on phenolic amines. These phenolic acids are apparently derived from amino acids by the action of aromatic amino acid decarboxylase and, in some cases, DOPAmine-β-oxidase. Small quantities of free or conjugated *p*-tyramine, *m*-tyramine, octopamine (the β-hydroxylated derivative of tyramine) and a number of other phenolic amines can be detected in normal human urine (23). There is a marked increase in their excretion when monoamine oxidase inhibitors are administered (24). In animals, inhibition of monoamine oxidase leads to the accumulation of octopamine and other amines in several tissues (25).

In addition to natural amino acids, several α-methylamino acids can be decarboxylated and subsequently β-hydroxylated *in vivo*. Following administration of α-methylDOPA or α-methyl-*m*-tyrosine, Carlsson & Lindqvist (26) were able to demonstrate accumulation of α-methylnorepinephrine or metaraminol, respectively, in the brains of rabbits. Porter & Titus (27) showed that rats given radioactively labeled α-methylDOPA excreted α-methylDOPAmine, 3-methoxy-α-methylDOPAmine, and products of the oxidative deamination of these amines, indicating that decarboxylation is an important metabolic reaction of the administered compound. Because of the quantitative importance of O-methylation, only a relatively small fraction of the amino acid is converted to α-methylnorepinephrine.

A variety of phenylethylamine derivatives are converted to the corresponding β-hydroxylated derivatives *in vivo*. Following administration of radioactive tyramine, labeled octopamine is rapidly formed *in vivo* (28, 29). The conversion does not occur in chronically sympathetically denervated rat salivary gland, suggesting that β-hydroxylation is confined to sympathetic neurones. Tyramine can still be bound, in proportion to dosage, in the denervated organ, indicating the presence of extraneuronal binding sites. When monoamine oxidase is inhibited, conversion of administered labeled tyramine to octopamine in intact rat salivary glands is markedly enhanced (30). Accumulation of endogenous octopamine in cat salivary glands following administration of a monoamine oxidase inhibitor is also dependent upon an intact sympathetic innervation (30). In addition to tyramine, *m*-tyramine, α-methyltyramine, α-methyl-*m*-tyramine and α-methylDOPAmine are converted to corresponding β-hydroxylated amines (31–33).

Tyrosine hydroxylase, which converts tyrosine to DOPA, is also capable of hydroxylating other amino acids. Thus, phenylalanine is converted to tyrosine (34) and α-methyltyrosine, which is an inhibitor of tyrosine hydroxylase (35), can be converted to small quantities of α-methylnorepinephrine *in vivo* (36), presumably via α-methylDOPA.

Storage.—Shortly after the introduction of α-methylDOPA as a therapeutic agent for hypertension (37, 38), it was found that it resulted in a long-term decrease in the norepinephrine content of the brain and other tissues (39, 40). When Carlsson & Lindqvist (26) found that α-methylnorepinephrine or metaraminol is present in the brains of rabbits given α-methylDOPA or

α-methyl-*m*-tyrosine, they suggested that norepinephrine depletion was a consequence of catecholamine displacement from its storage sites.

While appearance of α-methylnorepinephrine quantitatively corresponds to the disappearance of norepinephrine after administration of α-methyl-DOPA (26, 41, 42), the stoichiometric displacement of norepinephrine by metaraminol (26, 43, 44) has been disputed by some workers (45, 46). Anden (47) explained the discrepancy by showing that metaraminol is poorly extracted unless large volumes of strong acid are used. When large volumes of perchloric acid or trichloroacetic acid are used, stoichiometric replacement of norepinephrine by metaraminol or α-methylnorepinephrine can be demonstrated throughout the period of its depletion (47). The observations above indicate that the norepinephrine storage sites in sympathetic neurones are not specific. The D-isomer of metaraminol is not retained by the heart of rats and does not cause catecholamine depletion. This suggests that the α-hydroxyl group is involved in the binding, but that only the L-isomer is retained by the storage site.

Particles containing norepinephrine have been separated from the splenic nerve (48), brain (49), heart (50, 51), and other tissues (52). Norepinephrine-H^3, taken up by sympathetic neurones, is associated with the dense-core vesicles seen on electron microscopy (53). These particles can be separated with the microsomal fraction of tissue homogenates by centrifugation in a sucrose density gradient (52, 54). Musacchio et al. (55, 56) studied the subcellular distribution of phenylethylamine derivatives having one or two of the three hydroxyl groups of norepinephrine. When necessary, β-hydroxylation was prevented by the administration of disulfiram, which inhibits this reaction but does not interfere with norepinephrine binding (56, 57). When β-hydroxylation of tyramine, *m*-tyramine or α-methyltyramine was prevented, these amines were not retained by the particulate fraction. About 38 per cent of DOPAmine and about 50 per cent of the norepinephrine present in homogenates was retained in the particulate fraction (57). Similar results were obtained when α-methylDOPAmine and α-methylnorepinephrine were studied (58). Only about 30 per cent of the phenolic β-hydroxylated derivative, whether octopamine, *m*-octopamine or α-methyloctopamine, was retained. The results suggested that while β-hydroxylation contributes to the efficiency of binding, the catechol group is also important. *In vivo*, the ability of drugs which deplete norepinephrine to deplete false transmitters appeared to parallel the firmness of binding of the amines by the particulate fraction. Musacchio et al. (55, 56) suggested that more easily released norepinephrine may be bound at sites where only the β-hydroxyl or catechol group is involved.

Carlsson & Waldeck (59) studied the structure-activity relationships required for release of C^{14}-octopamine by phenylethylamines *in vivo*. It was found that 3-hydroxylation and L-β-hydroxylation enhanced potency; but no significant differences in the ability of DOPAmine, norepinephrine, octopamine, and tyramine to deplete this amine were found. They suggested that

other factors, such as metabolic stability, or ability to enter the sympathetic neurones, may play a role in obscuring differences in activity of the administered amines. The selective release of octopamine by tyramine, when equal amounts of octopamine and norepinephrine are present in the sympathetic nerves of the heart, may be important in explaining tachyphylaxis to tyramine (60). A similar preferential release of the less firmly bound amine has been found for metaraminol (61). These findings are consistent with the view that there is more than one type of binding involved in catecholamine retention in vesicular binding sites.

The accumulation of β-hydroxylated phenylethylamine derivatives, which can displace norepinephrine at its binding sites when certain amino acids or amines are administered, is a consequence of the relative nonspecificity of the enzymes involved in norepinephrine biosynthesis and of the nonselectivity of norepinephrine binding sites in the sympathetic nerve ending. Thus, the first criterion for a false transmitter, that the substance can be made to accumulate in norepinephrine storage sites, is satisfied by a variety of phenylethylamines.

Release.—The arrival of a nerve impulse at a nerve ending triggers release of a neurochemical transmitter. Little is known regarding the mechanism of transduction of neuronal membrane depolarization into transmitter release. There is a good deal of circumstantial evidence that synaptic vesicles, found in the region of synapses by electron microscopy, are preformed packets of transmitter. Katz (62) has reviewed the evidence that these vesicles provide the anatomical basis for the quantal release of acetylcholine responsible for miniature endplate potentials at neuromuscular junctions. Presumably, the almost simultaneous emptying of the contents of a large group of such vesicles following depolarization is responsible for the release of sufficient quantities of transmitter to elicit receptor activation.

A similar situation is thought to exist in the adrenergic nerve ending. This view is based on observations by Burnstock & Holman (63), who have provided some evidence for quantal release of norepinephrine from sympathetic nerve endings, and on the fact that dense-core synaptic vesicles found in adrenergic neurones appear to be associated with norepinephrine storage sites in adrenergic neurones (53). In the adrenal medulla, catecholamine release, stimulated by a variety of procedures, requires the presence of Ca^{++} (64), and is accompanied by release of stoichiometric quantities of adenine nucleotide (65). The latter observation is consistent with the hypothesis that during release, the entire content of the granule was discharged by a process similar to reverse pinocytosis (66). Further support comes from the demonstration that during such stimulation, protein, immunologically identical with that associated with catecholamine binding in the adrenal medulla, is released into the perfusing fluid in the same ratio to catecholamines as is present in the organ (67, 68). Since calcium ions also appear to be involved in norepinephrine release from the adrenergic nerve endings (69–71), the conclusions regarding release of catecholamine from the adrenal medulla have

been extrapolated to explain the process of norepinephrine release from sympathetic nerve endings. If such a process is accepted as the mechanism of transmitter release, it would be expected that amines which replace norepinephrine in its storage sites in synaptic vesicles will also be released by nerve impulses.

Following the demonstration that α-methylnorepinephrine and metaraminol are found in the brain when norepinephrine is depleted by administration of α-methylDOPA or α-methylmetatyrosine, Carlsson & Lindqvist (26) suggested that these amines replace norepinephrine and assume its functions. Day & Rand (72, 73) showed that α-methylDOPA could restore the actions of tyramine in reserpine-pretreated animals and suggested that α-methylnorepinephrine was formed and behaved as a false transmitter. Support for their view was obtained by the demonstration that such restoration of the action of tyramine does not occur when α-methylnorepinephrine formation is prevented by administration of disulfiram, a DOPAmine-β-hydroxylase inhibitor (74). Muscholl & Maitre (75) demonstrated that during stimulation of the cardio-accelerator nerves, α-methylnorepinephrine is released from the hearts of rabbits pretreated with α-methylDOPA. Crout et al. (76) showed that metaraminol, which had replaced norepinephrine in the hearts of cats, was also released during nerve stimulation. Their experiments provided the first direct demonstration that a foreign compound could replace norepinephrine and be released by sympathetic nerve stimulation.

Fischer et al. (77) examined the labeled compounds which appear in the effluent perfusate from isolated cat spleens during stimulation of the splenic nerve following treatment with tyramine-H^3, α-methyltryamine-H^3, *m*-tyramine-H^3, and α-methyl-*m*-tyramine-H^3. Only the β-hydroxylated derivatives of the amines were released as a consequence of nerve stimulation. Any increase in output of the precursor amines was related to splenic contraction (and consequent extrusion of platelets loaded with amines) and could be abolished by blocking splenic contraction with phenoxybenzamine. A β-hydroxylated product was also released following administration of amphetamine-H^3 (77); but it may have been α-methyloctopamine (parahydroxynorephedrine), since later studies by Thoenen et al. (78) showed that α-methyloctopamine accumulates in the spleens of cats pretreated with amphetamine and that it is released during splenic nerve stimulation.

When DOPAmine-β-oxidase is inhibited, administered DOPAmine-H^3 and α-methylDOPAmine-H^3 are not converted to their β-hydroxylated derivatives, but they can still be retained in the microsomal particulate fraction of tissue homogenates (33). Following accumulation of these compounds in the sympathetic nerves of the cat spleen, sympathetic nerve stimulation results in a diminished output of norepinephrine accompanied by release of the non-β-hydroxylated catecholamines (33, 79–81). The striking correlation between the degree of amine retention in the particulate fraction of tissue homogenates and the extent of amine release by sympathetic nerve

stimulation supports the view that vesicles are the site from which amines are released, but it does not rule out the existence of other binding sites (82).

N-methylation of norepinephrine occurs in the adrenal medulla, and the epinephrine formed is discharged into the circulation when the organ is stimulated. Epinephrine can be taken up, stored, and released from the sympathetic nerve ending by stimulation in the same manner as norepinephrine (83). The α-methyl analogue of norepinephrine can also be N-methylated; and the product, 3,4-dihydroxyephedrine, is formed in the adrenal medulla of rabbits treated with α-methylDOPA (84). 3,4-Dihydroxyephedrine can also be taken up, stored, and released by nerve stimulation in the same manner as α-methylnorepinephrine (85).

Guanethidine depletes norepinephrine stores in tissue (86, 87), is bound to some extent in the same particulate fraction as norepinephrine (88), and can be released by sympathetic nerve stimulation (89). Although this guanidine derivative may displace norepinephrine and have a role as a false transmitter (89), it interferes with sympathetic neuronal function prior to any decrease in catecholamine stores (90). Guanethidine may, therefore, diminish release of norepinephrine by interfering with the process of transmitter release rather than by replacing the transmitter.

Selective accumulation of a substance by sympathetic nerves and its release by nerve stimulation are not sufficient evidence to conclude that the substance is a false transmitter. Bretylium, a quaternary phenylethylamine derivative which was found to block the physiological effects of sympathetic nerve stimulation without blocking the effects of norepinephrine (91), accumulates in sympathetic ganglia and postganglionic sympathetic fibres (92). Although bretylium does not block the conduction of the nerve impulse down the axon (93), it does prevent norepinephrine release by sympathetic nerve stimulation (94). Bretylium-H^3 can be released from the perfused cat spleen by sympathetic nerve stimulation (95), but there are a number of differences between bretylium and norepinephrine which indicate that bretylium does not meet the criteria of a false transmitter. There is no evidence that bretylium can be stored at norepinephrine binding sites since, at doses which block release of norepinephrine, it does not deplete tissue catecholamines (90) and the labeled drug is not found in the same subcellular particles as norepinephrine (88).

Furthermore, acetylcholine, which will stimulate release of norepinephrine from the spleen, will not cause release of bretylium (95); and bretylium, which blocks release of norepinephrine, does not appear to block release of previously administered bretylium-H^3 (95). The arrival of the impulse at the nerve ending probably triggers a sequence of events which leads to the release of transmitter from storage sites in the vesicles. If the sequence involves the release of an intermediate quaternary amine, as suggested by Burn & Rand (96), or the disturbance of a binding site for quaternary amines at the neuronal membrane, then a compound which does not replace norepinephrine might be released during nerve stimulation (95).

Factors Altering Sympathetic Transmission in the Presence of False Adrenergic Transmitters

When a portion of the norepinephrine store has been displaced by a false transmitter, the efficacy of neurochemical transmission is influenced by a variety of factors. The presence of a substitute may diminish the quantity of physiological transmitter released, but it may also alter the effectiveness of the physiological transmitter and have a direct action on the receptor.

Diminished release of norepinephrine.—Replacement of norepinephrine by a false transmitter at the release site would be expected to diminish the norepinephrine released. Muscholl & Maitre (75) showed that while the total amount of pressor catecholamines released by sympathetic nerve stimulation from the isolated perfused hearts of rabbits pretreated with α-methylDOPA was unchanged, the amount of norepinephrine which could be determined by a fluorometric method was diminished. In the pithed rat, α-methylnorepinephrine was found to be equipotent with norepinephrine; but in the chemical determination, α-methylnorepinephrine exhibited only 3.8 per cent of the fluorescence of norepinephrine. Using this difference to estimate these catecholamines, they were able to show that the ratio of α-methylnorepinephrine to norepinephrine in the ventricle was the same as their ratio in the perfusate.

After inhibition of DOPAmine-β-oxidase with disulfiram, DOPAmine (which is formed from endogenous DOPA) and α-methylDOPA (which is formed from administered α-methylDOPA) can accumulate in the cat spleen (80, 81). DOPAmine and α-methylDOPA are not β-hydroxylated catecholamines and do not appear to replete the norepinephrine deficit. However, their ratio to norepinephrine released by nerve stimulation is the same as in the spleen (80, 81). Lindmar et al. (97) showed that the proportion of dihydroxyephedrine (α-methylepinephrine) or dihydroxypseudoephedrine to norepinephrine released from isolated perfused hearts of rabbits previously treated with these foreign catecholamines was the same as that found in the hearts after termination of the experiment.

Crout et al. (76) examined the release of metaraminol by nerve stimulation from the perfused cat heart at different times after its administration. In these studies, it was found that although levels of cardiac metaraminol were almost the same at two hours and at 17 to 20 hours, more metaraminol could be released in the shorter interval (76). It was, therefore, suggested that metaraminol taken up by the heart shifts from an "available" to a "less readily available" pool. If such shifts in distribution of false transmitters occur, then the extent of replacement of norepinephrine released by sympathetic nerve stimulation need not be directly related to the total replacement of norepinephrine.

Norepinephrine synthesis is thought to be controlled by a feedback inhibition mechanism which depends upon the levels of free norepinephrine in the sympathetic neurone (98). During displacement of norepinephrine from its binding sites, the level of free norepinephrine presumably increases, and its synthesis is inhibited during the period of time that excess amines are

present (99). Once depleted, however, the levels of norepinephrine in the cytoplasm diminish and synthesis again proceeds at a normal rate (99).

Newly synthesized norepinephrine appears to be preferentially released (unpublished observations). This might result in the retention of a previously formed false transmitter in a less readily available store. Synthesis and release of norepinephrine in the available store would then proceed at the usual rate, which is consistent with the observation of Anden (100) that urinary excretion of norepinephrine was normal after replacement of norepinephrine stores by metaraminol. Certain catechol compounds compete with the tetrahydropteridine cofactor of tyrosine hydroxylase (101). If such compounds are formed during false transmitter synthesis, then norepinephrine synthesis should be decreased. Muscholl & Rahn (102) found decreased norepinephrine excretion in patients being treated with α-methylDOPA. Thus, false transmitters may influence the amount of norepinephrine available for release by altering catecholamine synthesis.

When a train of impulses is delivered to the nerve ending, the relationship of response to frequency follows a sigmoid curve. With replacement of norepinephrine by a less efficient false transmitter, the curve would shift to the left. At rapid rates of stimulation, however, as more norepinephrine is released and the responses tend towards their maximum levels, the effects of rapid-rate stimulation may not appear significantly altered. Furthermore, at rapid rates of stimulation, new synthesis rather than mobilization from a less readily available store may play an increasingly important role in maintaining transmitter release. The new synthesis would result in an apparently preferential release of norepinephrine, and the presence of the false transmitter would be less apparent.

Consistent with this view is the observation of Thoenen et al. (103) that inhibition of norepinephrine synthesis by α-methyltyrosine diminishes the quantity of norepinephrine released from the perfused cat spleen by splenic nerve stimulation, even though relatively large quantities of the catecholamine remain in the spleen. Furthermore, Spector (104) reported that inhibition of tyrosine hydroxylase produces sedation and partial inhibition of the response of the cat nictitating membrane to nerve stimulation without complete depletion of norepinephrine stores, while Anden & Magnusson (43) could find no change in the response of the nictitating membrane when norepinephrine stores were almost completely replaced by metaraminol.

Alterations of response to norepinephrine.—The presence of foreign amines may alter the response to norepinephrine by changing the rate of its inactivation or altering its efficacy at the receptor. There is considerable evidence, previously reviewed by Gillespie (105) and Ferry (106), that the action of norepinephrine released by the nerve impulse is terminated largely by reuptake into the sympathetic nerve ending. The reuptake process, which serves to conserve transmitters, is competitively inhibited by a wide variety of phenylethylamine derivatives as well as other compounds (107, 108) and is evidently less specific than norepinephrine storage in vesicles. A number of

amines, some of which are false adrenergic transmitters, have been shown to be taken up by the sympathetic nerves by a process which is inhibited by cocaine and other known inhibitions of norepinephrine uptake (44, 109, 110). This process presumably plays a role in conserving false, as well as physiologic, transmitters in the sympathetic neurones. Thus, when uptake is blocked by protriptyline, metaraminol-H^3 disappearance is accelerated and the effects of reserpine on its depletion are potentiated (111). Although it does not appear likely that sufficient quantities of false transmitters are released by nerve stimulation to produce extraneuronal concentrations which competitively reduce the rate of uptake of simultaneously released norepinephrine, the presence of false transmitters in the sympathetic neurones of cats has been associated with enhanced responses to administered norepinephrine (112, 113). This potentiation of the catecholamine may be a consequence of reduced uptake.

In high doses (1 mg/kg), metaraminol causes subsensitivity of the blood pressure response of reserpine-pretreated spinal cats to norepinephrine (114). It was suggested that occupation of the receptors by metaraminol limits the number of receptor sites available for activation by the administered catecholamine. Although high doses of metaraminol are required to elicit this effect and it seems unlikely that the quantity of false transmitters released at the nerve ending could have a significant effect in blocking receptors, the concentration of transmitter in the synaptic cleft is not known.

Direct effects of false transmitters on receptors.—The relative potencies of sympathomimetic amines in activating adrenergic receptors can be evaluated only when their indirect effects are excluded by eliminating the norepinephrine stores with reserpine, by chronic sympathetic denervation or by antagonizing with cocaine (115–117). The direct actions of most amines which have been shown to be false adrenergic transmitters (e.g. octopamine, α-methyloctopamine, *m*-octopamine, metaraminol, α-methylDOPAmine) are several orders of magnitude less potent than norepinephrine in causing increases in blood pressure, contraction of the nictitating membrane or elevation of cardiac rate in the cat (115). This is probably equally true in most other organs and other species. The α-methyl analogues of norepinephrine, however, are fairly potent. The blood pressure of the pithed rat (41, 75, 118) and the cardiac rate of the anesthetized spinal cat (119) are equally sensitive to norepinephrine and α-methylnorepinephrine. The nictating membrane and the blood pressure of the cat (118, 120) are more reactive to norepinephrine than to its α-methyl analogue, but the reverse is true at certain other receptors, e.g. the uterus (118, 119) and bronchiolar muscle (120). In man, *d,l*-α-methylnorepinephrine is less than one-fourth as potent as norepinephrine (121).

The effect of α-methylepinephrine on the isolated rabbit heart is one-half to one-third that of norepinephrine (97), but the former compound causes a transitory drop in blood pressure which is blocked by β-blocking agents (85).

Thus, false transmitters may have actions opposite to those of the physiologic transmitters.

In summary, the net effect of the presence of a false transmitter in the norepinephrine storage site may depend on the sum of its effects on the rate of norepinephrine release, inactivation and interaction with the receptor, as well as any direct action of the false transmitter on the receptor. From these considerations it is possible, at least in theory, to explain how the presence of a false transmitter may reduce, not affect, or potentiate sympathetic responsiveness.

Functional Significance of Norepinephrine Replacement by False Transmitters

As a result of the factors discussed above, as well as factors which have not yet been identified, the consequences of administration of substances which result in replacement of norepinephrine stores may vary with the individual compound, organ, species, rate and time of stimulation, etc. Extensive observations of the functional significance of false transmitter accumulation have been made for only a few of the false transmitters, and these will be discussed individually.

Physiological consequences of α-methylDOPA administration.—Originally introduced into therapy because of its inhibition of decarboxylation of aromatic amino acids, α-methylDOPA was found to be an effective antihypertensive drug (37, 38, 122). It was soon recognized that its mode of action was not inhibition of decarboxylation, and it was proposed that the formation of α-methylnorepinephrine, the false neurochemical transmitter, was responsible for the hypotensive effects of α-methylDOPA (72, 73). A hypotensive action of α-methylDOPA has been found in conscious dogs (123) and in unanesthetized normal (124–126) and immunosympathectomized rats (127) made hypertensive with corticoids.

In spite of its hypotensive action in intact animals and in man, α-methylDOPA does not always interfere with responses to adrenergic nerve stimulation. Thus, Day & Rand (73) found that previous treatment with α-methylDOPA did not decrease the response of the rabbit ileum or the guinea pig vas deferens to sympathetic nerve stimulation. Treatment with α-methylDOPA does not prevent the pressor response to stimulation of the central end of the vagus in anesthetized dogs (128), the cardioacceleration produced by rapid (10–20/sec) stimulation of the stellate ganglion of rabbits (75, 129) or cats (130), nor does it diminish the normal tone of the nictating membrane in cats and dogs (123). It does, however, diminish the responses to low frequencies of sympathetic stimulation of the cardioaccelerator nerves in the dog (131) and the nictitating membrane of the cat (73).

In a detailed study of the effects of duration of treatment and the interval between the last dose and the experiment, Haefely et al. (113) found a complete lack of correlation between replacement of the physiological transmitter

by α-methylnorepinephrine and the impairment of the effects of sympathetic nerve stimulation in the cat spleen, although the ratio of α-methylnorepinephrine to norepinephrine released by nerve stimulation was correlated with that in the whole organ. They found that the sensitivity of the nictitating membrane to norepinephrine increases with duration of pretreatment with α-methylDOPA, but that four hours after its administration regardless of the pretreatment schedule, there is a depression of supersensitivity. The return towards normal sensitivity is required to unmask the functional consequence of release of the less potent false transmitter. Although the mechanism is still unknown, these findings may partially explain the apparent discrepancy between the effects of sympathetic nerve stimulation and the potency of the released transmitters.

Physiological consequences of α-methylmetatyrosine administration.—The product of decarboxylation and subsequent β-hydroxylation of α-methylmetatyrosine is metaraminol. It is a false transmitter, considerably less potent than norepinephrine, so that replacement of the physiological transmitter with metaraminol might be expected to produce a greater sympathetic deficit than does replacement with α-methylnorepinephrine. Anden & Magnusson (43) studied the effects of combined administration of α-methylmetatyrosine (400 mg/kg, i.p. daily for two days) and metaraminol (0.2 mg/kg, four hours before) on various parameters of sympathetic function in the rat and cat. They found that although 95 per cent of the norepinephrine in the heart had been depleted and displaced by metaraminol (or *d*-epinephrine there was no ptosis in rats, no nictitating membrane relaxation or miosis in cats. The blood pressure responses of the cats to tyramine, atropine plus carbocholine, splanchnic nerve stimulation or bilateral carotid occlusion were in the normal range. Stimulation of the cervical sympathetic chain elicited normal responses of nictitating membrane contraction, pupillary dilation and proptosis. It was, therefore, concluded that the major part of the store of adrenergic transmitter is probably not essential for normal transmitter function.

In contrast to these observations, Haefely et al. (112) found that previous treatment with α-methylmetatyrosine (100 mg/kg, twice daily for three days subcutaneously) markedly reduced the effect of sympathetic nerve stimulation on the cat nictitating membrane; the frequency/response curve shifted to the right by a factor of 4.7. In these animals, however, the heart rate was only slightly decreased and no change in blood pressure was found.

The antihypertensive actions of α-methylmetatyrosine which have been reported in man (132) are no greater than those of α-methylDOPA. Crout et al. (133, 134) have shown that orally administered metaraminol accumulates in the tissues (presumably the sympathetic neurones) and is effective in lowering blood pressure of hypertensive patients. In experimentally hypertensive rats, both metaraminol and α-methylmetatyrosine result in a dose-dependent lowering of blood pressure (126).

Other false adrenergic transmitters.—Although there are a variety of sympathomimetic amines which can release norepinephrine from its stores (135, 136) and form false adrenergic transmitters, the pharmacological effects of chronic administration of these amines have not been intensively studied. Ohler & Wakerlin (137) reported that chronic administration of paredrine (α-methyltyramine) or its N-methylated derivative was effective in the treatment of experimental hypertension in dogs, and Gill et al. (138) found that paredrine partially blocks sympathetic function in man. Paredrine is a substrate for DOPAmine-β-oxidase (19) and is converted to α-methyloctopamine in man (139) and animals (30). The latter compound is a false adrenergic transmitter and may be the mechanism of producing sympathetic blockade.

Monoamine oxidase inhibition.—Diminished sympathetic responsiveness, which develops with chronic inhibition of monoamine oxidase (140), is accompanied by the accumulation of octopamine in the sympathetic nerves (23, 30), This amine is a false transmitter (77), and it has been proposed that its accumulation and release, in place of a portion of the norepinephrine normally released, is responsible for the antihypertensive action of monoamine oxidase inhibitors.

False Adrenergic Transmitters as Pharmacological Tools

Almost as soon as it was discovered that some phenylethylamine derivatives were synthesized, stored, released, and transported by the same processes as catecholamines, it was realized that differences in metabolism made them useful tools for studying the adrenergic neurones (141). Investigators took advantage of the fact that α-methylamines are not substrates for monoamine oxidase (142) and that phenolic compounds are not methylated by catechol-O-methyl transferase (143). These compounds have been particularly useful in the study of transport and storage processes in the sympathetic neurone. The neuronal membrane of the sympathetic nerve is able to concentrate norepinephrine in the neurone by an active transport process which has been variously called "membrane transfer site" (144), "cell-membrane pump" (145) or "uptake" (146). Once inside the neurone, the unbound norepinephrine can be bound to particles: "storage sites" (144), "storage granule complex" (145) or "storage vesicles" (147). If the storage mechanism does not function, monoamine oxidase destroys the intracellular norepinephrine (148).

Metaraminol is taken up and stored in sympathetic neurones in the same manner as norepinephrine, but it is not susceptible to destruction by monoamine oxidase. In an effort to distinguish between drug effects on uptake and storage processes, Carlsson & Waldeck (145, 149–151) have used metaraminol-H^3 levels at different times after its administration to distinguish between blockade of the membrane pump and the storage granule complex. If storage is blocked, metaraminol-H^3 can still be concentrated in the neurone

by the membrane pump and shortly after its administration (15 to 30 min), metaraminol concentrations are normal. The leakage in the absence of retention by the storage granule complex causes the levels of metaraminol to fall more rapidly and to be reduced at later (3 hrs) times. If only the membrane pump is blocked, then initial uptake is diminished. If both the membrane pump and storage granules are blocked, release of metaraminol is more rapid than with drugs which block only the pump (protryptyline) or only storage (reserpine).

Giachetti & Shore (152, 153) distinguish between membrane transport and intracellular storage by using both metaraminol and *m*-octopamine, the latter being susceptible to destruction by monoamine oxidase. If membrane transport is inhibited, uptake of both metaraminol and *m*-octopamine is decreased. If storage is inhibited, but not transport, then uptake of metaaminol is normal. However, *m*-octopamine which is taken up and not stored is destroyed, and the result is apparent preferential inhibition of *m*-octopamine accumulation.

The α-methyl analogues of various phenylethanolamines have provided a means for assessing the role of monoamine oxidase in adrenergic neurones. They are retained for longer periods than the corresponding analogues which are substrates for monoamine oxidase (30, 44), they are not as readily depleted by reserpine (30, 44, 154), and their rate of disappearance is not slowed by monoamine oxidase inhibitors (30, 44). These observations support the previous views regarding the intraneuronal role of monoamine oxidase and are consistent with the expected effects of monoamine oxidase inhibitors (148).

In addition to being valuable pharmacological tools, the false adrenergic transmitters have been used to assess structure-activity relationships in binding and release and, as discussed earlier, have provided further evidence that the storage vesicle has binding properties similar to the site from which the physiological transmitter is released.

Conclusion

The nonspecificity of enzymes involved in the synthesis of norepinephrine and of the physical-chemical processes governing its storage, release, and transport can result in the accumulation of compounds, not normally present in the sympathetic neurone, which replace the physiological transmitter. Such false adrenergic transmitters may have additional actions which alter the effectiveness of the physiological transmitter, or they may have independent actions on the adrenergic receptor. Their effectiveness in altering adrenergic transmission also varies with the dosage and interval of administration of the drug which results in false transmitter accumulation and with the organ or species examined.

Because false adrenergic transmitters behave similarly to norepinephrine, the variety of chemical-structural modifications which alter affinity to binding sites involved in the physical-chemical processes or diminish susceptibil-

ity to enzyme action provide a useful means for examination of the effects of drugs on the storage, release, transport, and metabolism of norepinephrine in adrenergic nerves.

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