Adrenergic mediators

Mark Nickerson

The only adrenergic mediator of importance in the control of the mammalian vascular system is noradrenaline, and its predominant effect is vasoconstriction, mediated by adrenergic α receptors. This vasoconstriction varies in degree depending on the vascular bed, the type of vessel, the degree of sympathetic innervation, and the physiologic state. Adrenaline released into the blood stream plays a negligible role in vascular responses. Present-day investigations of adrenergic control of the circulation, particularly studies in man, depend heavily on the use of drugs as pharmacologic tools. Agents are now available which are purported to act by causing the release of noradrenaline from endogenous stores, blocking tissue receptors, preventing release from or depleting sympathetic nerve endings of the mediator, inhibiting the synthesis of the mediator or causing displacement by a "false mediator," and preventing the loss of the mediator by uptake and storage in nerve endings. It is emphasized that none of these pharmacologic tools is entirely specific and that each response studied requires specific confirmation of a relationship between an assumed mechanism of drug action and an observed effect before valid conclusions can be drawn. Results with agents preventing release from or depleting nerve endings of the mediator are particularly difficult to interpret. Although they remain obvious theoretical possibilities, none of the currently available agents has been shown to produce its effects by inhibiting noradrenaline synthesis or by causing the production of a false mediator. Inhibition of monoamine oxidase (MAO) does not alter most adrenergic responses and inhibition of catechol O-methyl transferase (COMT) has only limited effects, indicating that enzymatic inactivation plays no more than a minor role in terminating the effects of noradrenaline.

Knowledge of the humoral transmission of effects of adrenergic nerves, most post-ganglionic sympathetic nerves, has a long history, which is replete with uncertainties and poorly substantiated hypotheses. Adrenaline was demonstrated in extracts of the adrenal gland and identified chemically at the turn of the century. In 1905 Elliott published an extensive and detailed comparison of effects of adrenaline and of sympathetic nerve stimulation, which showed a very close similarity between the two. However, there were sufficient discrepancies to prevent him from concluding that adrenaline is involved in the action of these nerves, although he had tentatively suggested this in an abstract the preceding year. In 1921 Otto Loewi finally provided direct experimental evidence for the humoral transmission of autonomic nerve effects, and reported the release of a "Förderungssubstanz" from the frog heart, which he characterized fifteen years later as adrenaline. However, the well-known properties of adrenaline and the ascendancy of the theory of humoral transmission had already led to general acceptance of
this substance as the mediator at sympathetic nerve endings. This identification did not go unchallenged. As early as 1921 Cannon and Uridil7 had noted that sympathetic nerve stimulation released into the bloodstream minute amounts of a material with effects similar to but not identical with those of adrenaline. Cannon and his co-workers6 concentrated on the differences and developed a rather complex theory of adrenergic transmission involving combination of the nerve mediator with materials from other tissues to form sympathins E (excitatory) and I (inhibitory).

A new era in the study of adrenergic mediation was opened just 20 years ago when von Euler7 showed noradrenaline to be the predominant catecholamine in certain sympathetic postganglionic nerves. This demonstration, plus the earlier observation that sympathetic nerves of most sweat glands are cholinergic rather than adrenergic, reconciled most of the responses to sympathetic nerve stimulation with the known properties of the mediators. It also provided direct confirmation of the much earlier and often neglected observation of Barger and Dale8 that responses to sympathetic nerve stimulation are mimicked much more closely by responses to primary amines, including noradrenaline, than to secondary amines.

During the past 20 years noradrenaline has repeatedly been demonstrated to be the predominant catecholamine in and released by many mammalian adrenergic nerves, and it is widely accepted as the adrenergic transmitter. An exception of considerable historical interest is the fact that the predominant catecholamine in frog nerves is adrenaline, recently identified on the basis of both fluorescence microscopy and analysis of extracts.9 It thus appears probable that Loewi’s early characterization of the transmitter from the frog heart as adrenaline was correct.

There appears to be little doubt that the predominant adrenergic transmitter affecting the mammalian circulation is noradrenaline. Adrenaline is released into the bloodstream from the adrenal medullae in most situations in which sympathetic nerves are activated, but its effects on the vascular system are of minor importance. This is not because it is a less effective vasoconstrictor than is noradrenaline. Indeed, it is significantly more effective than the latter in most vascular beds. However, the amounts of catecholamine entering the circulation during sympathoadrenal discharge have much less effect on the vascular system than does the mediator released locally.10 Thus, a discussion of the vascular effects of adrenergic mediators is predominantly a discussion of the effects of noradrenaline. Qualitatively the effect is very simple, vasoconstriction. However, quantitatively the effects of adrenergic nerves vary widely with the vascular bed involved, the type of vessel, the extent of adrenergic innervation, and the physiologic state. Responses of the various types of blood vessels found in the eye have not been thoroughly investigated, and this area will not receive special attention in the present discussion, which will deal primarily with matters relevant to adrenergic transmission at all loci, particularly the effects of drugs which modify it.

Drugs affecting transmission at adrenergic nerve endings have increased tremendously in number and variety in recent years and are important to an understanding of sympathetic nervous system function because they are used extensively in studies of its effects, particularly in man, in whom a more direct approach is often not practicable. Although members of a single group of compounds may have opposite effects and agents with similar over-all effects may have entirely different mechanisms of action, one generalization is appropriate to all drugs which affect adrenergic mechanisms. None is entirely specific, i.e., all have more than one action. Consequently, demonstration of a mechanism of action and observation of a response does not justify the inference that they are cause and effect. Each such assumption requires direct confirmation, and, unfortunately, this
is lacking at present for many important phenomena.

Adrenergic receptors

A rational discussion of drugs affecting responses to adrenergic mediators requires an understanding of the types of receptors involved in these responses. Many recent studies have confirmed and extended the work of Ahlquist, which indicated that there are two major types of adrenergic tissue receptors, designated as α and β. Alpha receptors subserve stimulation of smooth muscle and gland cells, and also relaxation of intestinal smooth muscle. Beta receptors are involved in relaxation of smooth muscle, including that of the intestine, and in cardiac stimulation. It should be noted that neither of these receptor types has a distribution which conforms to the previously employed distinction between excitatory (E) and inhibitory (I) responses; their distribution agrees in general with the two types of responses distinguished by Dale in the course of his studies on the ergot alkaloids. Metabolic responses to catecholamines tend to follow the pattern of β more than that of α receptor activation, but they have some characteristics of both and may involve a third distinct category of receptors.

From the standpoint of the over-all physiology of the mammalian organism, vasoconstriction mediated by α receptors is by far the most important response to adrenergic mediators. Activation of β receptors by substances such as adrenaline or isoproterenol can produce profound vasodilatation in skeletal muscle. However, the predominance of noradrenaline in sympathetic nerve endings and the limited contribution of circulating catecholamines to vascular adjustments minimize the importance of this response in the economy of the body.

All sympathomimetic agents, including adrenaline and noradrenaline, can activate both of the major types of receptors, but the ratio of the two actions can vary widely, e.g., phenylephrine and methoxamine act predominantly on α receptors, whereas isoproterenol acts predominantly on β receptors. Adrenaline very effectively activates both types. The activity of noradrenaline on α receptors is only slightly less than that of adrenaline. Indeed, noradrenaline often appears superficially to be the more effective activator of α receptors because, in a complex response such as an increase in arterial pressure, the observed effects are the algebraic sum of effects mediated by both α and β receptors, and the greater action of adrenaline on the latter may reduce the response attributed to α receptor stimulation. Although noradrenaline has little effect on most β receptors in smooth muscle, it quite effectively stimulates those of the heart, which suggests that there may be subdivisions of specificity within the major receptor types.

Indirect acting sympathomimetics

The major structural characteristics required for sympathomimetic activity were delineated by Barger and Dale in 1910. These workers studied both excitatory and inhibitory responses, but within each there appeared to be a relatively continuous gradation of activity which did not suggest that more than one mechanism of action might be involved. A real dichotomy was first observed in 1927 by Tainter and Chang, who noted that cocaine could suppress responses to tyramine while augmenting those to adrenaline. A short time later Burn and Tainter found that chronic postganglionic denervation produced similar modifications of responses to the two agents and postulated that adrenaline acts directly on smooth muscle, whereas tyramine acts on sympathetic nerve endings. Interest in these observations was renewed in 1957 when Carlsson and co-workers showed that responses to tyramine were depressed when tissue stores of catecholamines were depleted by reserpine, and it has now been adequately demonstrated that tyramine and a number of other non-catechol sympathomimetic amines can act
through the liberation of noradrenaline from adrenergic nerve endings. However, actual depletion of catecholamine stores as a result of this action is not marked except with extremely large doses, and it appears unlikely that exhaustion of available supplies of mediator can account for the phenomenon of tachyphylaxis. The sympathomimetics often are classified into three groups, those which act predominantly directly on tissue receptors (catecholamines), those which act both directly and indirectly (ephedrine, metaraminol, methoxamine), and those which act predominantly via release of noradrenaline (tyramine, amphetamine). However, it probably is more accurate to consider the sympathomimetics to have a wide spectrum of activities, varying continuously from predominantly direct to predominantly indirect. All agents probably have some direct and some indirect action. Adrenaline can displace noradrenaline from sympathetic nerve endings, and it is equally clear that amphetamine can act directly on tissue receptors, although in some cases these may not be the same as the classical adrenergic receptors.

Interpretation of responses to indirect acting sympathomimetics, particularly in man, is complicated by the fact that responses to them are dependent on but are not proportional to the tissue stores of catecholamines, which can be altered by prolonged administration of the sympathomimetic or by other drugs, and by their direct actions on tissue receptors, particularly those classically characterized as 5-hydroxytryptamine (5-HT, serotonin) receptors. All stores of noradrenaline in sympathetic nerve endings appear not to be equally available for release by sympathomimetics, and it is a general observation that responses to an agent such as tyramine are little affected until depletion of tissue noradrenaline is extensive, probably near the limit which can be achieved in man.

The interpretation of responses to indirect acting agents is further complicated by the fact that these “sympathomimetics” can act directly on 5-HT receptors. This was first indicated by the observations of Vane on rat stomach smooth muscle in vitro, and has since been confirmed by direct receptor protection experiments on a variety of tissues. Indeed, even adrenaline has been shown to act effectively on the same receptors as does 5-HT in some tissues. Because of these complicating factors, it is important that the results of any experiment in which indirect acting sympathomimetics are used as pharmacologic tools be interpreted conservatively, particularly experiments in man, where it is usually impossible to determine directly either the status of catecholamines stores in a particular tissue or the specificity of the receptors involved in the observed response.

Adrenergic blocking agents

Adrenergic blocking agents are natural or synthetic compounds which inhibit responses to adrenergic nerve activity and to sympathomimetic amines by combining with the same tissue receptors as do agonists, such as catecholamines. This block may be competitive or relatively irreversible (nonequilibrium), but in either case the antagonist effectively reduces the number of tissue receptors available for activation. It thus must block an equal proportion of all responses due to activation of a particular type of receptor, irrespective of the specific agonist involved. Superficial observations often suggest that this is not the case, e.g., doses of an α-adrenergic blocking agent which reverse to depressor the pressor response to adrenaline only partially reduce the response to noradrenaline. However, apparent quantitative differences in the level of blockade are in fact due to actions of the sympathomimetics which do not involve α receptors, the much greater action of adrenaline than of noradrenaline on vascular β receptors in the above example. The actions of the two agonists on α receptors are equally blocked.

It was mentioned above that sympa-
Adrenergic mediators

Aclrenergic mediators differ quantitatively in their relative effects on α and β receptors. The blocking agents, however, appear to make a clear qualitative distinction between the two. This has been studied particularly in the myocardium, where exposure to phenoxycbenzamine at least 10,000 times that required to block vascular smooth muscle α receptors is without effect on β receptors.28 The adrenergic blocking agents are, therefore, highly specific tools for use in determining the nature of the receptors involved in adrenergic responses of various tissues. However, cognizance must be taken of the fact that certain β receptor blocking agents (dichloroisoproterenol, pronethalol) may themselves produce sympathomimetic effects due to an intrinsic activity on β receptors, and that a number of α receptor blockers (haloalkylamines, benzodioxans) may produce similar effects by releasing noradrenaline from sympathetic nerve endings. In addition, many of the α adrenergic blocking agents can block 5-HT receptors and some also inhibit responses to histamine.

Antiadrenergic agents

Antiadrenergic agents may be defined as drugs which inhibit responses to sympathetic nerve stimulation and to indirect acting sympathomimetics without blocking or even while enhancing responses to injected catecholamines. The effects of antiadrenergic agents thus appear to be predominantly on sympathetic nerve endings; the adrenal medullae are essentially unaffected by agents other than the Rauwolfia alkaloids, and these organs are relatively resistant even to reserpine. The antiadrenergic drugs appear to act by two major mechanisms which may both be involved in the effects of a single compound. Agents such as bretylium29 appear to act predominantly by inhibiting the release of noradrenaline in response to usually effective stimuli, perhaps by an action on the nerve cell wall. However, after prolonged administration some depletion of tissue (nerve ending) stores of catecholamines may contribute to the effect. Others, such as reserpine, appear to act predominantly by depleting the tissue stores of catecholamines. Both of these actions may contribute to the effects of guanethidine, the former being more prominent shortly after its administration and the latter during prolonged treatment. However, the fact that prevention of release can alone produce the major effects of this agent makes it difficult to assess the contribution of catecholamine depletion.30,31 Only limited data are available on tissue catecholamine levels in man after tolerated doses of reserpine or guanethidine,32 but these appear to be in the range where only a limited reduction in the release of noradrenaline in response to nerve activity or to indirect acting sympathomimetics would be expected. It is quite clear that agents such as methamphetamine can effectively antagonize the postural hypotension induced by guanethidine in man.33

Unfortunately, the actions of the available antiadrenergic agents are not limited to inhibition of the effects of adrenergic nerves with consequent relaxation of vasoconstrictor tone. Sympathomimetic effects, particularly marked with guanethidine, may be produced during the early phases of their action. These have been assumed to result from the release of endogenous catecholamines, but this action has been difficult to demonstrate consistently, and direct actions may be involved.31 Preoccupation with the endogenous amine-depleting action of agents such as reserpine and guanethidine has obscured observations which suggest other more direct actions. Reserpine administered intra-arterially has been noted to produce vasodilation in both normal and sympathectomyed extremities in man,34 and doses commonly employed in laboratory animals have been reported to depress several parameters of myocardial function,35-37 and to produce generalized myocardial damage and congestive heart failure in cats.38 Similarly, guanethidine has been reported to produce vasodilation in animals previously depleted.
of catecholamines by reserpine, an effect which may be due to a direct action on adrenergic β receptors. Although direct effects of these agents have been inadequately studied, they cannot be ignored. The antiadrenergic drugs would be unique among pharmacologic agents if all of their effects were due to a single mechanism of action.

The use of antiadrenergic agents as tools in the study of adrenergic control of the vascular system is made particularly difficult by the fact that these drugs can markedly sensitize to the effects of catecholamines. Guanethidine can cause an acute increase in sensitivity, even in tissues previously depleted of catecholamines by reserpine, but the major effect is very similar to that due to sympathetic postganglionic denervation. It reaches a maximum in 10 to 14 days and is greater for noradrenaline than for adrenaline. Responses after administration of an antiadrenergic agent are the resultant of decreased release of mediator and increased sensitivity of effector cells to it. Indeed, with appropriate doses and durations of guanethidine administration, and frequencies of nerve stimulation, it is possible to obtain augmented rather than depressed responses. Similarly, responses to indirect acting sympathomimetic amines after guanethidine have been reported to be less than, equal to, or greater than those of the control.

**Inhibition of mediator synthesis**

Inhibition of the synthesis of noradrenaline should provide a mechanism by which the available mediator and thus the effectiveness of adrenergic nerves might be reduced. Considerable effort has been expended in attempts to obtain drugs with this mechanism of action, particularly through inhibition of dopa decarboxylation or dopamine β-hydroxylation, but the agents studied to date have not been shown to produce their cardiovascular effects by inhibition of enzymes involved in mediator synthesis. Methyldopa has been most thoroughly studied for this possible mechanism of action and provides a good example of the pitfalls encountered when a mechanism of action is assumed before the basic pharmacologic properties of a drug have been adequately investigated. This compound was shown by Sourkes in 1954 to be an effective inhibitor of dopa (aromatic L-amino acid) decarboxylase in vitro, but attracted major attention only after its hypotensive properties were noted during studies of aromatic amino acid metabolism in man. The enzyme inhibition initially appeared to provide a very satisfactory explanation for the cardiovascular effects: Methyldopa reduced the synthesis of dopamine, which led sequentially to decreased synthesis of noradrenaline and perhaps adrenaline, depletion of tissue stores of catecholamines, depression of vasomotor responses, and a reduction in blood pressure. Consequently, attention was focused on the "biochemical" approach to its mechanism of action to the detriment of more classical pharmacologic analysis. Unfortunately, every step in the postulated sequence of events has been questioned by more recent observations.

Methyldopa effectively inhibits the decarboxylation of both dopa and 5-hydroxytryptophan in vivo and decreases tissue levels of dopamine, 5-HT, and noradrenaline. Reductions in the first two amines are transient, and the concentrations return toward normal roughly in parallel with reversal of the decarboxylase inhibition, but the noradrenaline levels remain depressed much longer. A causal relationship between decarboxylase inhibition by methyldopa and depletion of tissue catecholamines was called into question by the observation that the corresponding α-methylamine, which does not inhibit decarboxylation, depletes noradrenaline in much the same way as does the parent amino acid. It is known that methyldopa is itself decarboxylated in vivo to form α-methylamphetamine and subsequently α-methylnoradrenaline, which can be stored in the body and released in response to sympathetic nerve stimulation. However,
noradrenaline and its α-methyl congener are essentially equieffective transmitters, and it appears that this displacement by a “false transmitter” cannot explain the cardiovascular effects of methyldopa. Up to 95 per cent displacement of noradrenaline by much less active sympathomimetic amines does not produce appreciable functional impairment.8 This conclusion is supported by the observation that the marked reduction in drug decarboxylation induced by its hydrazine derivative does not reduce the hypotensive effect of methyldopa.9 Indeed, the role of any effect on catecholamine metabolism in the cardiovascular effects of methyldopa is questioned by the observations that the excretion of catecholamine metabolites is not much altered by hypotensive doses of the drug, that α-methyl-D-tyrosine depletes tissue catecholamines even more effectively than does methyldopa, but does not appear to be hypotensive, and particularly by the much neglected observation that adrenergic nerve function can be normal when the pressure-lowering effect of this agent is maximal.50 Methyldopa can effectively inhibit the pressor effect of dopa by preventing its decarboxylation to the pharmacologically active dopamine. At the present time this may be the only pharmacologic effect of the drug which can be clearly attributed to decarboxylase inhibition.

Inhibition of enzymatic inactivation of noradrenaline

Discussion of possible effects of agents inhibiting the destruction or inactivation of catecholamines on adrenergic transmission requires a distinction between termination of the action of the mediator and its ultimate destruction or inactivation; only the former is significant in determining the magnitude and duration of an effect. Adrenergic responses are much slower than cholinergic and it appears that physical dissipation of the transmitter, probably in part by movement back into nerve endings for restorage, is adequate to explain termination of the response in most cases; enzymatic destruction appears to play a relatively minor role. Ultimate chemical inactivation of adrenergic transmitters is achieved predominantly through methylation by catechol O-methyl transferase (COMT).51 Deamination by monoamine oxidase (MAO) is much less important, probably at least in part because adrenaline and noradrenaline are relatively poor substrates for this enzyme. Many very effective monoamine oxidase inhibitors have been developed during the past 15 years, but they do not have important effects on most adrenergic responses which can be attributed to MAO inhibition. Even in the presence of relatively complete inhibition of MAO, responses to sympathetic nerve stimulation or to injected noradrenaline are little affected, although responses to an agent such as tyramine, which is rapidly deaminated, are considerably augmented and prolonged. Similarly, deuterium substitution, which prevents attack by MAO, greatly potentiates responses to tyramine, but does not affect those to noradrenaline.52 A number of MAO inhibitors lower blood pressure and produce postural hypotension. However, these effects are the opposite of those expected to result from inhibition of an enzyme involved in terminating the effects of the adrenergic transmitter, and probably are due to actions other than inhibition of MAO. These agents can increase the catecholamine content of some tissues,53 and may increase or decrease the amount of catecholamines released from perfused organs during sympathetic nerve stimulation.54, 55 It has been suggested that a bretylium-like effect is involved, but the mechanism(s) are far from clear.

Considerable evidence has accumulated to indicate that in most situations O-methylation is a much more important pathway of catecholamine inactivation than is deamination,51 and inhibitors of O-methyl transferase can increase certain responses to catecholamines.56 However, these effects are not dramatic and it appears doubtful that inhibition of any enzyme system in-
Involvement in the inactivation of catecholamines can be expected to have a major effect on sympathetic nerve function.

Inhibition of uptake of mediator into storage sites

Catecholamines in the extracellular fluid can enter tissue storage depots, where they can be held for considerable periods of time and released in response to the usual stimuli. This movement can represent either net uptake of amine or exchange for previously stored catecholamines. However, the physiologic and pharmacologic significance of the two processes is entirely different. Net uptake of free noradrenaline could be an important factor limiting both the magnitude and duration of responses to this mediator, whereas exchange of one molecule of noradrenaline for another should have no effect on the observed response. Unfortunately, the technique most commonly employed to study the "uptake" of catecholamines, the appearance of injected \(^3\)H-noradrenaline in tissue stores, cannot differentiate between these two processes.

A number of drugs with quite diverse pharmacologic effects and mechanisms of action have been noted to decrease the movement of free noradrenaline into storage depots in tissues. This inhibition could involve a number of different mechanisms, some of which are suggested by other properties of the agents in question, e.g., limitation of passage through cell membranes by cocaine or bretylium, alteration of binding sites by agents such as reserpine or guanethidine which deplete tissue stores of catecholamines, etc. At least a part of the uptake into nerves is an active process which can transfer catecholamines against a considerable gradient, but the suggestion that reserpine may cause depletion and prevent the "uptake" of catecholamines by inhibiting this process does not appear to be correct.

Most drugs which decrease the appearance of \(^3\)H-noradrenaline in tissue depots also sensitize to the effects of catecholamines, and it has been postulated that the two are causally related, although in most cases it has not been shown that net uptake rather than exchange has been inhibited. The decreased "uptake" by storage depots is assumed to leave a higher concentration of free catecholamine to interact with tissue receptors for a longer period of time. This mechanism cannot be excluded in some drug-induced supersensitivity, but its involvement has not been firmly established either qualitatively or quantitatively. The close similarity between sensitization by some drugs and by denervation strongly suggests that other important mechanisms may be involved in the development of the supersensitivity.

Almost all of the endogenous noradrenaline in most tissues is contained in adrenergic nerve endings and disappears after section and degeneration of the postganglionic neurones. Following degeneration both the net uptake of noradrenaline and the appearance and retention of \(^3\)H-noradrenaline in tissues are greatly decreased. Inasmuch as net uptake has been shown to be drastically reduced in this situation, it provides the most cogent available case in which supersensitivity might be due to reduced loss of catecholamine into storage depots. However, it is well known that in organs with a dual innervation similar sensitization to catecholamines may follow section of either adrenergic or cholinergic neurones, and the effect of the latter is not explicable on this basis.

REFERENCES