BLOCKADE OF THE ACTIONS OF ADRENALINE AND NORADRENALINE[1]

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In 1905 Dale published his classical paper "On Some Physiological Actions of Ergot" (21) which contained a remarkably complete and accurate description of the pharmacological activity which will be referred to herein as adrenergic blockade. This consists of inhibition of many, but not all, responses to adrenaline, noradrenaline and other sympathomimetic amines and to sympathetic nerve activity. This field of investigation attracted the attention of many pharmacologists during succeeding years, but it is noteworthy that during the next forty years very few important observations regarding the basic characteristics of this type of pharmacological action were made which were not defined with reasonable clarity in this early publication. Important contributions during this interval included the demonstration and separation of the several alkaloids responsible for the adrenergic blocking activity of ergot (114) and observations that several classes of synthetic compounds possess qualitatively similar actions (8, 65, 77).

More recently, renewed activity in this field has led to considerable clarification of the loci and mechanisms of action of various groups of adrenergic blocking agents, and to the introduction of several new types of agents, some of which are sufficiently specific and nontoxic to allow effective blockade in man. Only a relatively small part of the more recent work can be covered here. Attention will be given to types and mechanisms of blockade and to differentiation between those responses to adrenergic stimuli which are blocked and those which are not, with particular reference to the use of adrenergic blocking agents in the analysis of physiological and pharmacological problems.

One of the major values inherent in the development of new pharmacological agents is their usefulness in the solution of various problems in physiology and pathological physiology. However, such applications require a precise understanding of their actions and limitations, and in particular, a clear recognition of the basic principle that *no drug achieves absolute specificity*, a concept too often overlooked in drawing conclusions from experiments in which pharmacological agents are employed.

A. *Types and mechanisms of blockade*

We may assume that activation of a cell by adrenaline or noradrenaline involves a primary combination of the stimulant with some cell constituent which then activates a chain of events or reactions of undetermined length and nature, culminating in the measured response. Antagonists which interfere with the

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first step, the reaction of agonist with specific receptors, usually exhibit the greatest specificity of action. Most compounds acting at this site appear to be in mass-action equilibrium with the receptor and the blockade produced is a measure of competition between the agonist and antagonist for receptor occupancy. Such agents will be referred to as *classical competitive antagonists.*

In the past it has been customary to assume that blockade at specific receptors always involves reversible, mass-action equilibria with the receptors. However, these two features are not necessarily associated. Recent studies have demonstrated at least two classes of blocking agents which combine with the same receptors as the agonist, as in classical competitive antagonism, but which then react with the receptor or some adjacent group to form a relatively stable chemical bond. This reaction precludes further mass-action "competition," and effectively reduces the number of available receptors (80). Such agents are best described as *nonequilibrium antagonists.*[2] The blockade produced has been referred to also as "irreversible competitive" (38) and "unsurmountable" (41). However, the blockade is not strictly irreversible, and the term unsurmountable is appropriate only when the antagonist is used in sufficiently large doses to prevent a maximal response even in the presence of massive amounts of agonist.

A third type of blockade is produced by agents which act at some point between the receptor and the ultimate response, the *noncompetitive antagonists.* Noncompetitive agents have received considerably less attention than competitive and nonequilibrium agents, largely because they tend to exhibit much less specificity of action.

Data on drug antagonism have been subjected to various types of quantitative treatment designed to distinguish between competitive and noncompetitive action (40, 64), and even to determine such details as the dissociation constant of the drug-receptor complex. The formulations of Lineweaver and Burk (64) and of others have been used to advantage in dealing with relatively simple enzyme systems. However, their application to even the simplest preparations involving responses of intact cells to agents such as adrenaline and noradrenaline requires reliance on a number of unproved assumptions, including the following: 1) the reaction between a drug and its receptors is reversible and obeys the laws of mass action; 2) all receptors are equally accessible to the drug; and 3) the response is proportional to the number of receptors occupied, *i.e.*, the maximal response occurs when all receptors are occupied.

Professor A. J. Clark, a pioneer in the quantitative analysis of drug action, clearly pointed out the unproved nature and even the improbability of a number of these postulates (15). However, this warning has been forgotten by many who have followed his lead in the use of calculations based on these assumptions.

[2] Only two groups of agents have been shown definitely to produce nonequilibrium blockade—the organophosphorus anticholinesterase agents (DFP, TEPP, etc.) and the β-haloalkylamine adrenergic blocking agents and antihistaminic agents [phenoxybenzamine (Dibenzyline) and congeners]. It is possible that hydrazides such as iproniazid (Marsilid) which inhibit monoamine oxidase may represent a third group with this type of action, but the studies necessary to establish such a mechanism have not yet been reported.

The results of such calculations usually have appeared reasonable when applied to the classical competitive blocking agents, largely because no independent data were available for comparison. However, when the same formulations were applied mechanically to the results of experiments with the β-haloalkylamine adrenergic blocking agents and antihistaminics (12, 13, 43), it became apparent that something was wrong. Such data were plotted to show that these agents are in competitive mass-action equilibrium with the receptors, whereas the results of several independent lines of investigation indicated quite clearly that they react to form a stable bond with the receptor or some adjacent structure (52, 77, 82).[3]

This discrepancy required explanation and one of the most logical places to look for error was in the postulates upon which the classical formulations of drug antagonism were based. The most important of these postulates for the interpretation of drug-receptor interactions is that the response is proportional to the number of receptors occupied and that a maximal response occurs only when all receptors are activated. This postulate implies that occupancy of receptors is the limiting factor in the observed response, and is fundamental to all attempts to treat drug-receptor interactions as analogous to adsorption isotherms (15, 55, 63). However, this assumption recently has been tested by independent methods and found to be untenable. Both indirect deductions from the dose-response relations and interactions of a series of substituted trimethylammonium compounds (113), and more direct studies of responses to adrenaline (38) and to histamine (80) of tissues in which various proportions of receptors had been inactivated, have demonstrated that occupancy of only a small percentage of the total number of receptors in normal smooth muscle is required for a maximal response. This finding is quite in keeping with current knowledge of other physiological systems which usually allow a considerable margin of safety. It immediately made obsolete previous methods of characterizing blocking agents and has left a gap in methodology which has not yet been adequately bridged.

No single clear-cut test is available to differentiate classical competitive from nonequilibrium blockade of tissue responses to adrenaline and noradrenaline. Determination of the concentrations (or doses) of agonist required to produce a standard response in the presence of varying concentrations of antagonist provides suggestive but not conclusive information. A straight-line relationship in plots of agonist:antagonist is characteristic of the classical competitive agents, whereas lines describing the action of nonequilibrium agents curve toward the agonist axis (82). However, analysis of such plots is complicated by the fact that, because of the excess of tissue receptors, a nonequilibrium antagonist can

[3] The suggestion that the prolonged action of the β-haloalkylamines is due to accumulation in and slow release from fat depots rather than to the formation of stable chemical bonds (6, 10) cannot be accepted in the light of present evidence. Very little accumulation in fat can be demonstrated when the usual blocking doses of these agents are administered *in vivo* (56) and experiments involving cross circulation and local intraarterial injection (3, 44, 58) and observations on the duration of blockade *in vitro* (37, 80, 81) provide direct evidence that the prolonged action is due to some effect of the drugs which can occur shortly after administration and which is only slowly reversible.

shift the dose-response curves over a considerable range without changing their slopes or asymptotes, thus producing a straight-line relationship in a plot of the type under discussion. The greater the range of doses or concentrations over which a straight line is obtained, the greater the assurance that one is dealing with a classical competitive antagonist. However, no precise statement can be made regarding the range required to "prove" such a mechanism of action, and all agents ultimately will cause a shift toward the agonist axis due to nonspecific "toxic" actions of the agonist, the antagonist or both.

Establishment of equilibrium between a classical competitive antagonist, agonist and receptors appears to be rapid. An old observation is that responses to concentrations of adrenaline and acetylcholine, adequate to produce a response in the presence of ergotamine or atropine respectively, are of essentially normal rapidity (14, 39, 73). This suggests that dissociation of the antagonist-receptor complex is not a significant factor in limiting the rapidity of the response. However, the older experiments did not take into account the fact that only a small percentage of the total receptors is required to produce even a maximal response to most agonists, and it is possible that occupancy of unblocked receptors was responsible for the rapid response in some cases. More recent studies have shown that prior treatment with atropine slows the response to certain alkyl-trimethylammonium compounds which must occupy a large percentage of the total receptors in order to produce a response (113). However, this delay, presumably related to the time required for an adequate number of atropine-receptor complexes to dissociate, is still very much shorter than the duration of action of atropine.

The failure of classical competitive antagonists with a duration of action of several hours to affect appreciably the rate of response to adequate concentrations of agonist, the fact that the presence of agonist does not facilitate washing these antagonists from isolated tissues (14), and the observation (38) that the rates of onset and dissipation of the blockade produced by a variety of classical competitive antagonists are equal, provide strong evidence that the duration of blockade by these agents is not dependent upon slow dissociation from receptors (*cf.* 34). These observations all emphasize diffusion to their site of action as the major factor determining the chronology of the action of these agents. The relatively long duration of action of many of them strongly suggests that passage through more than just extracellular space is involved, *i.e.*, that the receptors are not on the cell surface, but are located within the cell or at least within the substance of a cell membrane with appreciable thickness. This possibility has not yet been adequately explored.

Although nonequilibrium blocking agents tend to have a prolonged action, duration of action itself is not unequivocal evidence of type of blockade. Some β-haloalkylamines, such as N,N-dimethyl-β-phenyl-β-chloroethylamine (SKF-638A), have a relatively short action *in vivo* (32), although the characteristics of the blockade produced are very similar to those of Dibenamine or Dibenzyline blockade (32, 82). Conversely, some classical competitive antagonists such as atropine and ergotamine produce relatively prolonged blockade. However, duration of action is controlled by different processes in the two groups. Among

nonequilibrium agents it is dependent upon the stability of the chemical bond formed with the receptors, whereas among classical competitive blocking agents it appears to be determined by rate of diffusion to and from the site of action (biophase) (30, 33, 38). This differential offers a possibility of tests to distinguish between the two types of blockade, but has received only limited attention in this regard (87).

A final observation which contributes to the differentiation of classical competitive and nonequilibrium antagonists is that the removal of the former from isolated tissues by washing or from enzyme solutions by dialysis is a continuous function of the concentration gradient whereas dissipation of the latter is discontinuous. This difference is dependent on the fact that at least all known nonequilibrium antagonists (organophosphorus anticholinesterase agents and β-haloalkylamines) react with receptors in two steps. The first is a reversible "adsorption," the second a more stable chemical reaction (75, 80, 85). A portion of the blockade is dissipated relatively rapidly, a measure of the escape of loosely bound inhibitor from the biophase, whereas the remainder disappears much more slowly, limited by the rate of dissociation of the receptor-inhibitor complex. The second, stable component of the blockade increases with time of drug-enzyme or drug-tissue incubation (75, 82).

Although observations on a series of the factors mentioned above may differentiate classical competitive from nonequilibrium antagonism, they do not differentiate the latter from noncompetitive antagonism. However, the characteristic two steps in the action of nonequilibrium antagonists provides a basis for direct determination of whether the blockade involves the specific agonist receptors. Because the antagonist is in mass-action equilibrium with the receptors during the first stage, the presence of agonist will decrease the degree of blockade of specific receptors, which can be tested after removal of both the agonist and the unreacted antagonist. Such studies have demonstrated that both the anticholinesterases diisopropylfluorophosphate (DFP) and tetraethylpyrophosphate (TEPP) (5) and the β-haloalkylamine blocking agents (37, 79, 80) do in fact react with specific receptors in the establishment of blockade. That the degree of inhibition of the blockade is proportional to the number of receptors occupied by agonist is suggested by the observation that it is linearly related to the logarithm of the concentration of agonist (79). The specificity of such "receptor protection" tests is indicated by the fact that where an antagonist is effective against responses to several types of stimulants (adrenergic, cholinergic, histamine, etc.), the presence of agonist during exposure to the blocking agent inhibits the blockade of responses to only one specific type of agonist (37).

A similar procedure can be employed to demonstrate that classical competitive antagonists react with specific receptors. Once the specific locus of action of a nonequilibrium agent has been established by "receptor protection" tests with the agonist in question, a classical competitive agent can be substituted for the agonist in similar experiments. If the nonequilibrium agent has a significantly longer action than the classical competitive agent tested, inhibition of blockade by the latter can be demonstrated, and provides direct evidence of the locus of

blockade. Experiments of this type have been performed with DFP vs. physostigmine (eserine) or neostigmine (Prostigmin) (59) and with Dibenamine or Dibenzyline vs. benzodioxanes, imidazolines or ergot alkaloids (109; Nickerson, unpublished), and appear to provide more conclusive evidence of reaction with specific receptors than do the standard agonist:antagonist plots discussed above.

B. *Physiological characteristics of blockade*

Pharmacological studies of the adrenergic blocking agents and physiological investigations utilizing them as tools would be much simplified if one could assume that all responses to sympathomimetics and to adrenergic nerve activity were inhibited equally. Unfortunately, this is not the case. Different responses are blocked to varying degrees and the relative inhibition of various responses varies widely between different groups of blocking agents and even within a series of closely related compounds. However, a general pattern of activity applicable to the major actions of most blocking agents can be recognized and provides a useful baseline from which to evaluate the properties of individual compounds. Only the general pattern can be discussed here, and the reader must keep in mind that it does not agree completely with the properties of any one adrenergic blocking agent, and that *all available agents have important actions in addition to blockade of responses to adrenaline and noradrenaline.*

The effects of blocking agents can be discussed most conveniently in terms of the various actions of adrenaline and noradrenaline. Peripheral excitatory and inhibitory, cardiac, metabolic and central nervous system actions are all well known. Although the relative potencies of various sympathomimetics with respect to these actions differ widely, the differences are only quantitative and one can generalize that all sympathomimetics have some activity in each of these categories.

The adrenergic blocking agents separate more clearly the various types of adrenergic responses. Although the effects of adrenaline and noradrenaline on the heart are "excitatory," Dale's first study of the blockade produced by ergot revealed important differences between the excitation of smooth and of cardiac muscle (21). Consequently, reference to peripheral excitatory effects usually is limited to those on smooth muscle and exocrine glands, cardiac effects being classified separately. In addition, the cardiac effects of sympathomimetic amines can be further subdivided into physiological responses and pathological responses, the production of arrhythmias, on the basis of resistance or susceptibility to inhibition by the common adrenergic blocking agents.

A second type of classification, which has much to recommend it, was first proposed by Ahlquist (4). On the basis of the relative activities of a series of sympathomimetic amines in inducing various responses, he concluded that adrenergic receptors are of two types: α-receptors, which are involved in the excitation of smooth muscle (exocrine glands presumably have the same type of receptors), and β-receptors, which are involved in inhibitory responses of smooth muscle, except for the intestine, and in cardiac stimulation. Although this classification leaves many areas of adrenergic activity unexplored, it has proved to be

a useful approximation. Ultimately, some similar classification based on additional information regarding the nature of the tissue receptors involved may be expected to provide the most reliable categorization of the many responses to adrenaline and noradrenaline.

Excitatory responses of smooth muscle and exocrine gland cells. These responses are readily blocked by the classical adrenergic blocking agents, and their inhibition has for many years been tacitly accepted as a criterion for inclusion of an agent in this category of drug action. The general characteristics of the blockade and the relative effectiveness of various agents appear to be fairly constant when tested on a variety of structures giving this type of response, although a careful selection of agents and doses may provide considerable separation of responses in some circumstances (28). Isolated segments of blood vessels or perfused vascular beds, the radial fibers of the iris, some uteri, guinea pig seminal vesicles and several other structures have been used as test objects. In general, detailed quantitative analyses of the characteristics and mechanisms of action of blocking agents are most reliable when carried out on the simplest possible *in vitro* preparations. However, reversal of the pressor response to adrenaline in intact anesthetized animals is one of the most dramatic expressions of adrenergic blockade and has received major attention. Unfortunately, critical interpretation of the results of this type of test is not easy because cardiac, vasoconstrictor and vasodilator components are involved in a variable and unknown relationship to one another.

Differences in the ease and completeness of the reversal of pressor responses to various sympathomimetics, first noted by Barger and Dale in 1910 (7), frequently have been misinterpreted as indicating variations in the degree of blockade of their vasoconstrictor activity. However, the observed differences appear to be due primarily to differences in actions of the sympathomimetics in question other than the production of vasoconstriction, particularly their inhibitory, vasodilator actions (91, 117). Studies of the blockade of responses of the cat nictitating membrane, a structure with little or no inhibitory component in its response, to a series of sympathomimetic amines indicated that all are blocked effectively (91), and detailed studies of blood pressure responses to adrenaline and noradrenaline in cats in which adrenergic vasodilator responses had been minimized by pithing (36) or by a continuous infusion of isoproterenol (isopropylarterenol, isopropylnoradrenaline) (86) demonstrated that the excitatory, vasoconstrictor actions of these naturally occurring catecholamines are inhibited equally.

However, differences in the net cardiovascular responses to adrenaline and noradrenaline after partial blockade with various agents are sufficiently striking to be useful in differentiating between these catecholamines (29, 72, 86). Reversal of the pressor response to adrenaline and only limited reduction of that to noradrenaline by appropriate doses of the blocking agents is dependent on the fact that pressor or peripheral blood flow responses to small doses of adrenaline are reversed when only about 50% of the vasoconstrictor component is blocked (46, 86). This degree of blockade is adequate to allow the prominent vasodilator, depressor effect of adrenaline to determine the net response. However, noradrenaline elicits very little vasodilatation and consequently the effect

of the considerable residual constrictor response is recorded. This interpretation is strengthened by the observation that in fully blocked animals, reversal of the response to noradrenaline is most readily demonstrated with relatively large doses of the amine (91, 121), large enough to exert a significant inhibitory action.

It appears safe to generalize that excitatory responses to equieffective doses of all sympathomimetics are blocked to essentially the same degree. This conclusion might have been anticipated on the basis of the mechanism of action of the antagonists. Combination of the blocking agents with excitatory (α) receptors, either reversibly or by stable chemical bonding, will reduce the number available for activation and thus reduce the effectiveness of all adrenergic stimulants proportionately. It is true that differences in the affinity and intrinsic activity of different agonists will modify the number of residual receptors occupied and the degree of activation of the tissue. However, the contribution of these factors will not differ in the pre- and postblockade responses and therefore will be cancelled out in the selection of equieffective doses.

A second generalization is that all adrenergic blocking agents inhibit responses to circulating sympathomimetics more readily than those to adrenergic nerve activity, frequently observed in the difficulty in completely blocking cardiovascular reflexes. This difference has been accentuated in many cardiovascular studies by the comparison of responses to nerve stimulation with those to adrenaline rather than to noradrenaline. However, the relationship appears to hold even on structures with relatively pure excitatory responses (88, 101) and in direct comparisons with noradrenaline (88, 108, 123). The basis for this differential has not been clearly established, but it does not appear to be explicable on the basis of the earlier assumption (11) that blocking agents act at the surface of effector cells to prevent diffusion of circulating agonists to the receptors (88). The magnitude of the differential between ability to block responses to circulating mediators and those to adrenergic nerve stimulation varies considerably from one agent or group to another, being greatest for certain benzodioxanes such as piperoxan (933F, Benodaine). Although this agent can inhibit responses to both circulating catecholamines and to nerve stimulation (101), the former are blocked much more readily, a factor of considerable importance for its utilization in the diagnosis of pheochromocytoma (42).

Inhibitory responses. Inhibitory responses to adrenaline, noradrenaline and other sympathomimetics involve primarily smooth muscle. Inhibition of glandular secretion by adrenaline or adrenergic nerve stimulation frequently has been observed. However, vasoconstriction within the gland can severely limit secretory activity, and it usually is impossible to determine reliably the contribution of direct inhibitory effects on the glandular tissue, although direct, nonvascular adrenergic inhibition of gastric secretion by isoproterenol appears to have been demonstrated (50). The most prominent inhibitory responses are those of coronary and skeletal muscle blood vessels, bronchi, intestine, and the uteri of some species when under proper hormonal influences. In contrast to the consistent, predictable blockade of excitatory responses of smooth muscle, the blockade of inhibitory responses is seen irregularly, and conflicting reports are

common. A general review of the available observations indicates that blockade is reported most frequently with the less specific adrenergic blocking agents and on complex organs such as the isolated intestine (77). Inhibition of adrenaline-induced intestinal relaxation by the ergot alkaloids has been reported most frequently (76, 103, 104). The locus of this blockade has not been clearly identified. However, it has been shown that the effect is not prevented by hexamethonium and atropine, and occurs with concentrations of the blocking agents which do not significantly alter the relaxation induced by papaverine (104).

Intestinal relaxation in response to a series of sympathomimetic amines appears to follow a pattern more closely parallel to the α-receptor (excitatory) responses of smooth muscle than to other inhibitory (β-receptor) responses (4). It is possible that some difference between the receptors involved in this response and those responsible for most other smooth muscle relaxation may provide an explanation for the observed inhibition of intestinal relaxation. However, it is quite clear that the blockade of intestinal relaxation does not at all parallel the blockade of excitatory responses among the various groups of adrenergic blocking agents, a strong argument against its classification as an α-receptor response.

The only apparently inhibitory response blocked by Dibenamine and other β-haloalkylamines is suppression by catecholamines of the peristaltic reflex response to increased lumenal pressure in isolated intestine (71). This is a coordinated response of a complex organ, and it is probable that the catecholamines act primarily to inhibit transmission in intramural ganglia, as they have been shown to act on other, more accessible ganglia (70). The validity of this interpretation is strengthened by the observation that blockade of adrenaline suppression of the peristaltic reflex and of nicotine stimulation are parallel (71).

Blockade of adrenaline-induced relaxation of the uteri of various species has been reported occasionally, but most workers have confirmed the early observation of Dale (21), who found that relaxation of the nonpregnant cat uterus was not inhibited by ergot.

Specific inhibition of adrenaline-induced vasodilatation has not been clearly demonstrated with any of the classical adrenergic blocking agents either *in vivo* or *in vitro*. Apparent inhibition of vasodilatation in isolated vascular beds (45, 119) appears always to require very high doses of blocking agent, administered intraarterially, and it is probable that the subsequent failure of adrenaline to induce vasodilatation is due either to the fact that the vessels in question are already maximally dilated or to a nonspecific depression of the vascular smooth muscle. The specificity of this "blockade" has not been tested in most of the reported studies. However, one careful comparison has been made of the inhibition by Dibenzyline, tolazoline (Priscoline), phentolamine (Rogetine, Regitine) or azapetine (Ilidar) of vasodilatation induced in dog skeletal muscle by adrenaline, noradrenaline, or nerve stimulation (cholinergic as shown by its inhibition by small doses of atropine) (123). This study demonstrated a parallel inhibition of the adrenergic and cholinergic vasodilatation, indicating that no specific blockade of the adrenergic response had been produced. Inhibitory

responses of vascular smooth muscle are not blocked *in vitro* even by very high concentrations of antagonist (37), nor is relaxation of tracheobronchial smooth muscle inhibited except by very high concentrations which also suppress aminophylline-induced relaxation (2).

In contrast to the variable and often unconvincing reports of blockade of adrenergic inhibitory responses by the classical adrenergic blocking agents, a new type of agent [1-(3′,4′-dichlorophenyl)-2-isopropylaminoethanol (DCI; dichloroisoproterenol), the dichloro derivative of isoproterenol (Isuprel)] specifically blocks a variety of inhibitory responses (97). Indeed, the blocking activity of this compound appears to be largely complementary to that of agents such as Dibenamine; it has very little effect on excitatory (α-receptor) responses.

In summary, it appears that no conclusive demonstration of direct, specific blockade of inhibitory responses of smooth muscle to adrenaline or noradrenaline by the classical adrenergic blocking agents has been made. Relatively nonspecific suppression of the reactivity of smooth muscle to a variety of relaxing agents can be produced by the adrenergic blocking agents, and probably by most other chemicals. A number of cases of apparent suppression of adrenergic inhibitory responses in complex organs have not yet been adequately explained, but even the frequently demonstrated suppression of adrenaline inhibition of intestinal motility and tone by the ergot alkaloids must represent a pharmacological action very different from that involved in the blockade of excitatory responses. The probability that this effect is dependent on actions other than a specific blockade of adrenergic inhibitory responses of the smooth muscle is strengthened by comparison with the clear-cut blockade of inhibitory responses produced by dichloroisoproterenol (DCI).

Cardiac responses. Responses of the myocardium to adrenaline and noradrenaline are separated clearly into two groups by the actions of the adrenergic blocking agents. These may be termed *physiological responses* (*e.g.*, positive chronotropic and inotropic responses) and *pathological responses*, the production of abnormal rhythms. Although the chronotropic and perhaps the inotropic responses of amphibian hearts may be inhibited under suitable conditions (35, 90), these responses of the mammalian heart are not effectively blocked by the classical adrenergic blocking agents. As in the case of inhibitory responses, a number of reports of blockade of the positive chronotropic and inotropic responses of mammalian hearts have been published (9, 110). Unfortunately, such reports rarely provide information regarding the specificity of the effect, *i.e.*, whether only responses to sympathomimetics were inhibited or whether responses to such diverse agents as the cardiac glycosides or calcium ions also were blocked.

The most recent reports of blockade of the positive inotropic response to adrenaline, noradrenaline and other sympathomimetics by the classical adrenergic blocking agents are found in a series of papers describing studies in which contractile force was measured by means of a strain-gauge arch sutured to the myocardium (16, 17). In this preparation, Dibenamine, Dibenzyline, phentolamine, piperoxan and azapetine appeared to inhibit the inotropic response. The doses required were considerably larger than those necessary to block excitatory

responses of smooth muscle, but after extensive or complete inhibition of the recorded response to sympathomimetics, a significant response to a cardiac glycoside was still obtained. These observations conflict with the results of a considerable number of earlier studies which failed to show any blockade of either chronotropic or inotropic responses by doses which did not markedly depress the myocardium (1, 58, 84, 96), and must be accepted with caution. It appears possible for a number of factors affecting diastolic filling and initial myocardial fiber length to influence the results obtained by this method so as to produce a spurious "blockade." A reevaluation of this problem employing much simpler test objects, atria of rats, rabbits and cats, and cat papillary muscles *in vitro*, and the blocking agents Dibenamine, Dibenzyline, tolazoline, piperoxan and Hydergine in maximal tolerated doses failed to show any clear inhibition of either chronotropic or inotropic responses to adrenaline (Nickerson and Chan, unpublished). In contrast, very low concentrations of dicholoroisoproterenol effectively antagonized both responses. It may be hoped that a more thorough study of the strain-gauge-arch technique will provide an explanation for these divergent results.

Cardiac arrhythmias induced by adrenaline and other sympathomimetic amines with or without associated sensitization by cyclopropane or other hydrocarbons are effectively inhibited by the classical adrenergic blocking agents both in animals (25, 92, 95) and in man (83). In general, the inhibition is parallel to adrenergic blocking activity, but in the case of some agents such as the benzodioxanes, which have a potent direct depressant action on the myocardium (22), it is probable that this property also is involved in the protection against arrhythmias. Part of the protective effect of adrenergic blockade is due to peripheral inhibition of the usual pressor response to the sympathomimetic amine (74, 89). An additional direct effect on the heart appears also to be involved (89), although this was not demonstrated in one series of experiments on the induction of arrhythmias in dog heart-lung preparations (31).

As might be expected, arrhythmias due to factors other than adrenergic stimuli are suppressed much less completely and consistently by the adrenergic blocking agents. The benzodioxanes have been shown to inhibit arrhythmias due to electrical stimulation or to barium chloride (23), but this undoubtedly is due to the strong quinidine-like activity of these agents (22). More specific agents have little effect on arrhythmias not involving adrenergic stimuli. Phentolamine does not alter arrhythmias induced by cardiac glycosides (19) or by mercurial diuretics (20), and Dibenamine does not increase the threshold for electrically-induced fibrillation (Nickerson, unpublished), except for a short period after the administration of large doses when a transient, direct myocardial depression occurs (1).

Inhibition of various types of arrhythmias by the more specific adrenergic blocking agents may provide useful, but not necessarily definitive, information regarding the participation of adrenergic factors in their genesis. Dibenamine does not significantly alter the development of arrhythmias following acute coronary occlusion (67), but phentolamine and particularly Dibenamine and Dibenzyline may effectively inhibit late postocclusion arrhythmias (51). Arrhyth-

mias of the latter type have been shown to be potentiated by sympathomimetic agents (66). The development of ventricular fibrillation during hypothermia is unaltered by adequate blocking doses of Dibenamine or SY-21 [SKF-501; N-ethyl-N-(9-fluorenyl)-β-chloroethylamine] (18).

Metabolic responses. The most prominent "metabolic" responses to sympathomimetic agents are liver glycogenolysis and the consequent increase in blood glucose concentration; muscle glycogenolysis, leading to lactacidemia; and a transient hyper- and more prolonged hypokalemia. Many other metabolic effects of administered adrenaline have been reported, but it often is unclear whether they are primary or secondary responses. Many of these responses, such as the over-all increase in metabolic rate, are very complex and the results of attempts to inhibit them with specific blocking agents are difficult to interpret.

Several of the adrenergic blocking agents effectively inhibit adrenaline-induced hyperglycemia. However, this effect does not parallel blockade of peripheral excitatory responses among the various groups of agents or even within a single series of closely related compounds such as the ergot alkaloids (53), which are the most effective inhibitors of this response. Actually, ergotamine appears to be considerably more potent than dihydroergocornine (DHO 180) in inhibiting the hyperglycemic response to adrenaline in both rabbits and cats, whereas the reverse is true with respect to the blockade of vasoconstriction. The inhibition of liver glycogenolysis thus appears to be an expression of a pharmacological property quite different from that involved in the blockade of excitatory responses of smooth muscle. This conclusion is supported by the fact that posterior pituitary principles, particularly vasopressin (Pitressin), and several antihistaminics without other evidence of adrenergic blocking activity also effectively inhibit this metabolic response (60).

Many important details of the sequence of events by which adrenaline induces glycogenolysis recently have been worked out by Sutherland and associates (98), but their data do not as yet allow conclusions regarding the step at which blockade may occur. If the blockade were competitive, one might reasonably assume that it occurred at the initial stage of direct adrenaline action, which appears to involve some particulate cellular material found in several of the fractions obtained by differential centrifugation. However, blockade of this response has not been shown clearly to be competitive and its inhibition by several compounds which do not affect other responses to adrenaline suggests that it may occur at some later point in the chain of events, a noncompetitive antagonism.

Glycogenolysis in skeletal muscle leading to lactacidemia appears not to be blocked effectively by any of the adrenergic blocking agents which have been studied in this regard (60). Blockade of this reaction has received only limited attention. It is not known why liver glycogenolysis is inhibited while skeletal muscle glycogenolysis is not. However, it is possible that this differential action of the blocking agents may provide a useful tool in the study of glycogenolysis. Conversely, demonstration of any major biochemical difference in the mechanism of adrenaline-induced glycogenolysis in the two loci would provide an important clue to the possible site of action of agents blocking liver glycogenolysis.

Although few observations have been published, the effect of adrenaline in mobilizing fat to the liver appears to be amenable to blockade. Ergotamine inhibits the development of fatty livers in rats treated with ergothionine (112), a response which has been shown to be dependent upon adrenaline.

The transient hyperkalemia which is induced by adrenaline primarily by a release of potassium from liver cells is effectively inhibited by the common adrenergic blocking agents (26, 27, 93). In contrast to the blockade of adrenaline-induced liver glycogenolysis (hyperglycemia), inhibition of hyperkalemia appears to parallel blockade of peripheral excitatory responses. Indeed, the ability of Dibenamine to block the hyperkalemia without affecting significantly the induced hyperglycemia provides the most convincing evidence that the release of potassium and of glucose from liver cells are not causally related (27). A more generalized effect of adrenergic blockade on potassium handling is indicated by the observations that Dibenamine increases tolerance to infused potassium by increasing its apparent "volume of distribution" (57), and that it blocks glucagon-induced release of liver potassium (27).

Central nervous system responses. The ability of adrenergic blocking agents to inhibit central nervous system responses to adrenaline and noradrenaline is quite unclear. This is due in part to lack of knowledge regarding the physiological role, if any, of these substances in the central nervous system, and the lack of any clear understanding of the mechanism by which these and other exogenously administered sympathomimetics produce their gross effects. The most prominent gross central effect of adrenaline, noradrenaline, and other sympathomimetics is stimulation, whereas the best studied local effect is inhibition of transcallosal synaptic transmission between optic cortices (69). These effects are not necessarily contradictory, as any combination of stimulation and inhibition within the central nervous system can be explained on the basis of the interaction of inhibitory and facilitatory areas. However, the possibility of such a relationship is no proof of its existence.

Efforts to demonstrate blockade of central stimulation due to exogenous sympathomimetics have produced negative or inconclusive results. A considerable number of adrenergic blocking agents has been shown to antagonize the increased motor activity induced by methamphetamine (desoxyephedrine, Methedrine) in the mouse (116). However, it is difficult to attribute this effect to specific adrenergic blockade. It is not quantitatively correlated with the peripheral blocking activity of the compounds studied, and adrenaline and noradrenaline themselves produce a similar antagonism of methamphetamine-induced activity. Dibenamine does not alter appreciably adrenaline-induced respiratory stimulation in animals (84) or in man (54).

It has been demonstrated that chlorpromazine (Largactil, Thorazine) blocks effectively adrenaline-induced inhibition of transcallosal synaptic transmission. However, it is difficult to attribute this effect to specific adrenergic blockade because reserpine (Serpasil) and azacyclonol (Frenquel), which have little or no adrenergic blocking activity in other tests, also are effective (69). Unfortunately, the effects of more specific and effective adrenergic blocking agents on this prepa-

ration have not been tested. In more complex tests it has been shown that relatively large doses of adrenaline can suppress a conditioned avoidance response in rats and that Dibenzyline completely prevents this loss (62). However, the authors probably are correct in attributing their results to a generalized weakness and lethargy due to peripheral actions of adrenaline and to the inhibition of these peripheral effects by Dibenzyline.

Recent demonstration of relatively high concentrations of noradrenaline in the brain stem (118) has greatly increased interest in the possible role of adrenergic mediators in normal and abnormal central nervous system function. Investigations predicated on the assumption that adrenergic blocking and ataractic activity would be associated (111) led to the development of a benzodioxane derivative (Ethoxybutamoxane) which is highly effective in laboratory tests. However, this agent has relatively weak adrenergic blocking activity and it cannot be accepted at this time that its effectiveness proves either that noradrenaline plays a role in abnormal emotional processes or that the agent is in fact active because of its adrenergic blocking properties. The benzodioxanes exert a number of effects on the central nervous system which are very different from those of other adrenergic blocking agents (77) (see below) and consequently it is difficult to attribute them to adrenergic blockade *per se*.

Some studies purporting to show central adrenergic blocking activity (112) are entirely dependent upon the assumption that an agent such as chlorpromazine can produce effects only by blocking responses to adrenaline or noradrenaline. This assumption probably is not valid for any drug, and it appears to be particularly dangerous in the case of a pharmacologically complex agent such as chlorpromazine.

Adrenergic blocking agents have been utilized by many workers in attempts to demonstrate a possible adrenergic link in the activation of various pituitary functions. However, many of the results have been inconclusive. It now appears to be quite clear that a specific adrenergic step is not involved in the release of adrenocorticotrophin (ACTH). Various adrenergic blocking agents have been shown to inhibit ACTH release induced by the injection of adrenaline or of other sympathomimetic amines, but these agents do not effectively block release in response to other types of stress (48, 94, 115). Where a small generalized reduction in the response to various types of stress was observed, the effect was produced also by 2-dibenzylaminoethanol (a hydrolysis product of Dibenamine), which does not produce adrenergic blockade (107).

The picture with respect to the release of gonadotrophic hormone is less clear-cut. Ovulation in the rabbit has been induced by the injection of small amounts of adrenaline or noradrenaline into the third ventricle, the hypothalamus or the adenohypophysis (24, 68). Several of the relatively specific β-haloalkylamine blocking agents have been shown to block the ovulatory response of the rabbit to adrenaline or noradrenaline injected into the third ventricle or to coitus, and to inhibit spontaneous ovulation in rats and fowl (68, 105, 124). The imidazoline blocking agents, tolazoline and phentolamine, were found to be ineffective. A

certain degree of specificity of this effect is indicated by the fact that 2-dibenzylaminoethanol, in doses which produce a comparable degree of direct central nervous system stimulation, is ineffective (105). The above observations suggest that an adrenergic link is involved in the release of gonadotrophic hormone and that the β-haloalkylamines may inhibit ovulation as a direct result of their adrenergic blocking activity. However, several disturbing observations remain to be reconciled with this hypothesis. These include the ineffectiveness of the potent adrenergic blocking agent phentolamine, even when administered in near-lethal doses, the fact that the β-haloalkylamines appear to be as effective one minute after injection as they are one or more hours later (106), and the observation that adrenaline and noradrenaline injected into the hypothalamus are much less effective in inducing ovulation when the solutions are carefully neutralized and the volume of the injection reduced (24).

It has been demonstrated that large doses of Dibenamine (60 to 80 mg/kg) or atropine (700 mg/kg [*sic*]) can block the milk letdown reflex in lactating rats (47). However, methylergonovine (Methergine, a semisynthetic ergot alkaloid devoid of adrenergic blocking activity in most tests) was found to be 30 to 80 times more potent than Dibenamine. In the aggregate, these data do not appear to provide a sound pharmacological basis for the conclusion that both cholinergic and adrenergic steps are involved in this process.

It appears that all adequately studied adrenergic blocking agents influence central nervous system activity. However, the observed effects do not form a really consistent pattern, nor can they be correlated qualitatively or quantitatively with blocking activity as determined on peripheral structures. The β-haloalkylamines produce mild sedation and feeling of lethargy (99), and Dibenamine can induce relatively specific derangements of time sense and memory illusions (54, 100). The latter have not been observed with Dibenzyline, which because of its greater adrenergic blocking potency has been administered to man in considerably smaller doses. The two compounds probably do not differ qualitatively in this regard, but it is at least clear that induction of these relatively specific psychic reactions does not parallel adrenergic blocking activity. When administered rapidly to animals in large doses, the β-haloalkylamines are effective convulsants, but this effect clearly is unrelated to adrenergic blockade (78).

Both the natural and dihydrogenated ergot alkaloids characteristically produce many prominent effects on the central nervous system (102). These usually can be observed with doses lower than those required to produce even minimal peripheral adrenergic blockade. A central inhibition of sympathetic nervous system activity (61) is responsible for any peripheral vasodilatation and fall in blood pressure which these agents may induce in man, and nausea and vomiting due to an action on the chemoreceptor trigger zone of the medulla (120) are prominent. In contrast to the ergot alkaloids, the benzodioxanes act centrally to increase sympathetic tone. They may thus induce a considerable rise in blood pressure in unanesthetized animals (49) and in man (42). Present evidence is

inadequate to show that any of the above effects can be attributed to "central adrenergic blockade," and their diversity argues in favor of attributing them, at least provisionally, to other properties of the drugs in question.

There is considerable need for a careful study of the blockade of central actions of adrenaline and noradrenaline on simplified systems where the responses can be recorded objectively. In the absence of such studies, one can conclude only that blockade of any central action of the sympathomimetic amines has not been demonstrated convincingly, and that the present inadequate evidence suggests that blockade of these actions probably is not a salient property of the currently employed adrenergic blocking agents.

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