THE RECEPTORS FOR EPINEPHRINE AND NOREPINEPHRINE (ADRENERGIC RECEPTORS)[1]

ROBERT F. FURCHGOTT

Department of Pharmacology, State University of New York, College of Medicine at New York City, Brooklyn, N. Y.

A. *The pharmacological classification of adrenergic receptors*

Dale, in his classical work on the influence of ergot alkaloids on the effects of epinephrine and sympathetic nerve stimulation (14), was the first clearly to differentiate two distinct types of receptors for epinephrine. One type, on which epinephrine acted to give excitatory or motor responses, was "paralyzed" by ergot alkaloids, whereas the other type, on which epinephrine acted to give inhibitory responses, was not "paralyzed." His results were unequivocal for most smooth muscle effectors. However, in the case of the heart, the ability of ergot alkaloids to block the two excitatory effects of epinephrine—the increases in rate and force—was not clearly demonstrated. Subsequent work over many years showed that none of a wide variety of natural and synthetic agents which block the excitatory effects of epinephrine in smooth muscle is capable of giving a clear-cut blockade of its excitatory effects in mammalian heart. [See Nickerson's review (48) for detailed references.] Thus, the excitatory receptors for epinephrine in mammalian heart can be pharmacologically differentiated from the excitatory receptors for this agent in smooth muscle.

Today we group the various receptors for epinephrine under the general heading of adrenergic receptors, *i.e.*, adrenoceptive sites. It is now well established that these receptors are the sites of action of norepinephrine and certain other closely related sympathomimetic amines as well as of epinephrine (2, 22, 24, 33). In recent years two noteworthy attempts have been made to modify and extend Dale's original classification of adrenergic receptors (Table 1). Ahlquist (1, 2) classified adrenergic receptors mediating specific responses in different effector organs largely on the basis of the order of potency of a series of five sympathomimetic amines (epinephrine, norepinephrine, isoproterenol, α-methylepinephrine and α-methylnorepinephrine) in eliciting thses responses. His classification, for which only two types of receptors (alpha and beta) were required, was further supported by the fact that responses mediated through alpha receptors (except possibly intestinal relaxation) could be blocked by adrenergic blocking agents, whereas responses mediated through beta receptors could not.

Lands (41, 42) objected to certain aspects of Ahlquist's classification. He preferred to classify adrenergic receptors in heart as undifferentiated (Acr) since "this organ is stimulated by substances with strong affinity for either" the excitatory receptors (Ac) or the inhibitory receptors (Ar) of smooth muscle.

[1] Part of the unpublished experimental work referred to in this review was supported by grants from the Life Insurance Medical Research Fund and the New York Heart Association.

Also, according to Lands, the receptors in intestinal muscle responsible for relaxation might better be put in the same class with inhibitory receptors in other smooth muscle organs. He criticized Ahlquist's use of order of relative potency of a series of sympathomimetic amines for classifying receptors, and pointed out that the relative potencies of different sympathomimetic amines on one type of effector organ often varied considerably with the species of animal and the experimental conditions used.

In view of this latter criticism of Lands, I thought it might be informative to compare the relative potencies of the levo-isomers of epinephrine (E), norepinephrine (NE) and isoproterenol (ISO) in producing responses in a series of isolated effector organs all from the same animal (rabbit) and all suspended in the same medium (Krebs-bicarbonate).[2] Isoproterenol was included partly

[2] Relative potencies of a number of agonists acting on a specific type of receptor in an effector organ are often taken as a measure of relative affinities of the agonists for the receptor. Such an interpretation of relative potencies, even in experiments with isolated organs, may sometimes be quite erroneous. Strictly speaking, affinity should be quantitated as the association constant (inverse of the dissociation constant) of the agonist and the receptor within the biophase of the effector cells (24). Since we cannot as yet measure concentrations of free agonist molecules, free receptor sites or receptor-drug complex within the biophase, we cannot at present quantitatively determine affinity. Of course, potency would generally be expected to increase with increase in affinity; but a number of other factors influence the relative potencies of agonists, and, theoretically, these factors may sometimes be more important than the relative affinities. One such factor would be the distribution coefficient, which under steady-state conditions in an isolated test system would determine the concentration of agonist in the biophase relative to that in the aqueous phase, as follows (24):

$$(D_b) = \frac{(D_a)\, k_a}{k_b + k_e},$$

where k_a is the rate constant for entry of the drug into the biophase from the aqueous phase, k_b is the rate constant for escape from the biophase, k_e is the rate constant for enzymatic inactivation in the biophase, (D_b) is concentration of the agonist in the biophase, and (D_a) is its concentration in the aqueous phase. Any increase in distribution coefficient due to changes in the rate constants determining it would increase the concentration of the drug in the biophase, and thus lead to an increase in potency. Thus, one agonist might have approximately equal or even less affinity for a given type of receptor than a second agonist, but the relative potency of the former might still be greater due to a markedly higher distribution coefficient.

A third important factor influencing relative potencies would be the capacity of each agonist, once it had reacted with a receptor, to activate the response in the effector cell (24). This capacity has been termed the "intrinsic activity" by Ariëns (3) and the "efficacy" by Stephenson (60). According to the latter's theory (using some different symbols):

$$S = e \times (RD) \text{ and}$$

$$A = f(S),$$

where S is the stimulus to the cell, e is the efficacy, (RD) is the concentration of receptor-agonist complex, and A is the response. From this formulation, it can be seen that potency would increase with efficacy; and it would be quite conceivable for one agonist with a lower affinity or lower distribution coefficient than another agonist to be more potent because of a higher efficacy.

TABLE 1
Proposed classifications for adrenergic receptors

Organ or Tissue	Response	Classification of Receptor	
		Ahlquist (2)	Lands (42)
Smooth muscle	Contraction	alpha	Ac*
Smooth muscle (except intestine)	Relaxation	beta	Ar†
Intestinal smooth muscle	Relaxation	alpha	Ar
Heart	Increase in rate	beta	Acr‡
Heart	Increase in strength	beta	Acr
Liver & skeletal muscle	Glycogenolysis	alpha	—

* Ac = excitatory receptor.
† Ar = inhibitory receptor.
‡ Acr = undifferentiated receptor.

because of its common use in experiments comparing relative potencies of catecholamines, and partly because it has been claimed by Lockett (43) to occur physiologically. Our results with these three catecholamines, as well as our findings on the susceptibility of the responses in question to blockade by Dibenamine and DCI [dichloro-analogue of isoproterenol; 1-(3′,4′-dichlorophenyl)-2-isopropylaminoethanol], are shown in Table 2. In the case of excitatory responses in the smooth muscle preparations (aorta, stomach and uterus)—responses mediated through receptors termed "alpha" by Ahlquist or "Ac" by Lands—the potency ratios of the three sympathomimetic amines are rather similar and in the order E > NE > ISO, and in each case the responses can be readily blocked by the adrenergic blocking agent Dibenamine. It is of some interest that with all three smooth muscle preparations used, ISO at very high concentrations was able to activate a contractile response.

In the case of the inhibitory responses in aorta and stomach and the two excitatory responses in atria, the order of potency is ISO > E > NE, but the actual potency ratios may vary considerably from one response to another. These differences in potency ratios might be used as an argument against the classification of the various receptors mediating the four responses in question under a single heading. However, the additional finding that DCI readily blocks all four responses indicates that the receptors responsible for these responses have some common chemical property; and thus, for the time being it would appear reasonable to classify both the smooth muscle inhibitory receptors, excepting those of intestine, and the cardiac excitatory receptors as beta receptors.

Our findings with the interesting new agent, DCI, agree well with the previous findings of Powell and Slater (49) on smooth muscle other than intestine, and of Moran and Perkins (46) and Dresel (15) on heart. It is the first agent which we have encountered which gives a clear-cut blockade [apparently by competitive antagonism (31)] of the inotropic and chronotropic actions of sympathomimetic

amines on the heart,[3] and of the inhibitory actions of these amines on all smooth muscles tested other than intestine. However, even this agent is not an ideal blocking agent, for the concentrations required for blockade often approach those which directly depress contractility in smooth and cardiac muscle. In addition, DCI appears to have some sympathomimetic activity on the very receptors which it blocks—the degree of activity depending on the particular effector organ on which it is tested. In Stephenson's modified receptor theory (60), DCI might be classified as a weak "partial agonist" rather than a true antagonist.

The responses of isolated rabbit small intestine to catecholamines and adrenergic blocking agents deserve special comment. As pointed out by Ahlquist (2), the potency ratio of E to ISO may vary significantly in the same intestinal strip during the course of a single experiment. Sometimes we have observed such variations but in atropinized intestinal strips we have usually observed no appreciable change in potency ratio over several hours (28). The inability of Dibenamine to block effectively the inhibiting effects of catecholamines on rabbit intestine has been long recognized (48). Confirming Rothlin *et al.*(54), we found with rabbit intestine that dihydroergotamine at a concentration of about 10^{-6} effectively blocked the inhibitory action of E, at a concentration of about 10^{-7}. However, the blockade was easily broken through by a 20-fold higher concentration of E; and the inhibitory action of this higher concentration was not in turn blocked by a 20-fold higher concentration of dihydroergotamine (26). This latter finding throws some doubt on whether dihydroergotamine, in blocking the inhibitory action of low concentrations of epinephrine on the intestine, is actually competing for adrenergic receptors in that structure.

Returning to the results of our experiments on rabbit small intestine shown in Table 2, it will be noted that the potency ratio of E, NE and ISO for the inhibitory response in this organ is considerably different from that for the responses in the other organs studied. Perhaps of even greater significance is the finding that neither Dibenamine nor DCI effectively blocked the response of the intestine to catecholamines under the same experimental conditions (concentration of agents, time of exposure, etc.) under which they blocked responses in other test organs. Thus, both the potency ratio and the lack of blockade with either agent might warrant the classification of the adrenergic receptors in rabbit small intestine as a type distinct from either alpha or beta receptors. With regard to the lack of blockade by DCI, however, a word of caution should be introduced. In testing the ability of this agent to block the inhibitory effects of catecholamines

[3] The work of Krayer and his associates on the antiaccelerator action of veratramine and certain other veratrum alkaloids on the heart (*e.g.*, see 37, 38, 39) clearly shows that these alkaloids can antagonize the chronotropic action of epinephrine on the heart without significantly altering its positive inotropic action. If this selective antagonism were due to the selective blockade of adrenergic receptors responsible for increase in heart rate, then these receptors would be clearly differentiated from those responsible for increase in force. However, the veratrum alkaloids in question decrease heart rate even in the absence of added epinephrine, and after the heart has been depleted of norepinephrine (36a). It would appear, therefore, that their antagonism to the chronotropic action of epinephrine is a "physiological" antagonism and not the result of blockade of adrenergic receptors (35, 36a).

TABLE 2

*Relative potencies of epinephrine, norepinephrine and isoproterenol on isolated tissues from rabbits**

Tissue	Response	Concn. of Epi. Required for Moderate Response	Relative Potency†			Susceptibility to Blockade by	
			Epi.	Norepi.	Iso.	DB‡	DCI‡
Thoracic aorta	Contraction	1–5 × 10^{-9}	1	1	~.01§	+	−
Stomach muscle (from fundus)	Contraction	2–10 × 10^{-9}	1	1	~.001§	+	−
Uterus	Contraction	5 × 10^{-8}	1	0.2–0.5	~.001§	+	−
Thoracic aorta after DB	Relaxation	1 × 10^{-8}	1	0.02	4	−	+
Stomach muscle after DB	Relaxation	1 × 10^{-8}	1	0.2–0.5	30	−	+
Atria (spontaneously beating)	Increased rate	1 × 10^{-7}	1	0.8	100	−	+
Left atrium (electrically driven)	Increased force	1 × 10^{-7}	1	0.8	200	−	+
Intestine (duodenal segment)	Relaxation	1 × 10^{-8}	1	1–2	2–5	−	−

* All tissues were suspended in oxygenated Krebs bicarbonate medium at pH 7.4. Temperature was 37°C except in case of atrial preparations for which it was 27°.

† Relative potency is based on determination of concentrations giving equal moderate responses (10 to 50% of maximal response with epinephrine). Levo-isomers of all drugs were usually used. In some experiments where racemic isoproterenol was used, concentration was calculated for levo-isomer only.

‡ DB = Dibenamine; DCI = dichloro-analogue of isoproterenol. Exposure to DB, at a concentration of 10^{-6} to 2 × 10^{-5}, was for 20 to 30 min. Exposure to DCI, at a concentration of 10^{-5} to 10^{-4}, was for 10 to 20 min. If either agent produced direct effects on the preparations used (such as depression or stimulation of activity), sufficient time was allowed after washout for these direct effects to disappear partly before tests for blocking activity were performed.

§ Contraction with isoproterenol was obtained at concentrations exceeding those necessary for maximal relaxation (27).

on rabbit intestine, the situation is complicated by the fact that DCI itself in concentrations of 10^{-6} and higher drastically depresses tone and contraction amplitude (28). This depression appears to be largely "nonspecific," although it may be due in part to the action of DCI as a partial agonist for adrenergic receptors. Following washout of the muscle chamber at the end of a 10- to 20-minute exposure to DCI (usually at 10^{-5}), recovery of tone and amplitude occurred so gradually, that usually 10 to 30 minutes had to be allowed before there was sufficient recovery to permit adequate testing of the catecholamines for inhibitory activity. There is some chance, therefore, that the delays in testing after washout were long enough to permit recovery from a DCI blockade which existed but could not be tested for during the actual exposure to this agent. It should be noted, however, that such a transient blockade would be inconsistent with the long persistent blockade with DCI following its washout from other smooth muscles and heart.

We have not investigated the potency ratio of catecholamines on the glycogenolytic response of isolated rabbit liver and skeletal muscle, or the ability of Dibenamine and DCI to block this response. The results of Ellis (18) and Sutherland and Cori (62) on rabbit liver slices indicate that the potency ratio for E:NE:ISO for glucose production is approximately 1:0.2:0.1–0.2. This potency ratio differs considerably from any of those reported in Table 2 for responses in other isolated organs of rabbit. Ellis *et al.* (19) also found that dihydroergotamine effectively blocked the glycogenolytic activity of epinephrine on rabbit liver slices. I am unaware of any reports on the ability of Dibenamine to block epinephrine-induced glycogenolysis in rabbit liver slices; however, Harvey *et al.* (34) reported Dibenamine to be only a very weak antagonist of epinephrine-induced hyperglycemia in the living rabbit, even though Dibenzyline was relatively effective. In conclusion, it must be admitted that the pharmacological evidence presently available does not justify our classifying the receptors through which catecholamines mediate glycogenolysis as either alpha or beta receptors.

B. *Location of adrenergic receptors in effector cells*

1. *Evidence for adrenergic receptors in cell membranes.* The evidence favoring the location of adrenergic receptors in cell membranes is largely electrophysiological. The electrical changes in intestinal smooth muscle accompanying the inhibition of mechanical activity by epinephrine have been studied extensively by Bozler (8) using wick electrodes, by Bülbring (11) using intracellular microelectrodes and by Burnstock (13) using the "sucrose-gap" method. The results of these investigations show that epinephrine decreases the electrical excitability, increases the "resting" membrane potential and abolishes action potentials (spike activity). These dramatic changes in the electrical characteristics of the cell membranes of intestinal smooth muscle on application of epinephrine strongly suggest that the adrenergic receptors mediating these changes are located within the cell membranes themselves; and that the reaction of epinephrine with such receptors alters the ionic permeability properties of the membrane in such a way as to produce hyperpolarization and abolish propagated action potentials, thus inhibiting mechanical activity in turn.

I am unaware of any studies with intracellular microelectrodes of the electrical changes produced by epinephrine in smooth muscles which are stimulated by this agent. However, the results of a number of studies with external electrodes afford valuable information. For example, the contraction of the nictitating membrane produced by epinephrine or sympathetic nerve stimulation is associated with membrane depolarization, slow waves and perhaps propagated action potentials (6, 16, 53). Also smooth muscle of the rabbit uterus, when activated by epinephrine, shows bursts of rapid potential changes with each sustained contraction (47). These changes in the electrical characteristics of cell membranes of smooth muscles which are stimulated in activity by epinephrine may be taken

as evidence that the excitatory adrenergic receptors of smooth muscle are also located in the cell membranes.[4]

Pacemaker cells in the sino-atrial node of heart also show characteristic changes in electrical activity in the presence of epinephrine. Recent investigations (36, 66) with intracellular microelectrodes show that epinephrine increases the rate of rise of the "diastolic depolarization," or "pre-potential" of these cells. This more rapid spontaneous depolarization leads to a more frequent triggering of action potentials and thus a faster heart rate. The alteration of the electrical characteristics of the membranes of cardiac pacemaker cells by epinephrine again points to the membrane itself as the site of adrenergic receptors—in this case, the receptors mediating the chronotropic action.

In the case of cardiac muscle cells in atria driven at a constant rate, records obtained with microelectrodes show that epinephrine and norepinephrine (58, 65) slightly prolong the repolarization phase of the action potential. However, this prolongation in itself seems unlikely to account for the increased strength of contraction, since comparable prolongation produced by decreased calcium or by ryanodine (59) actually leads to a decrease rather than an increase in strength. Thus, the results of electrophysiological experiments to date provide little evidence that adrenergic receptors mediating the inotropic action of epinephrine and norepinephrine are in myocardial cell membranes; but they do not necessarily exclude this possibility.

2. *Evidence for intracellular adrenergic receptors.* The strongest evidence for an intracellular location of adrenergic receptors is the well recognized glycogenolytic effect of epinephrine and closely related sympathomimetic amines in liver, skeletal muscle, cardiac muscle and certain smooth muscles. [See Ellis' review (18) for extensive references.] Since glycogenolysis depends on the activity of intracellular enzymes, it appears most likely that its stimulation is initiated by a reaction of epinephrine with receptors located intracellularly. The outstanding work of Sutherland and his colleagues has shown that the stimulation of glycogenolysis by epinephrine results from an activation of the enzyme phosphorylase (52, 61, 63, 67). This activation is not the result of an interaction of the enzyme and epinephrine but rather the result of an accumulation of cyclic

[4] It should be noted that the argument that the electrical changes of smooth muscle cell membranes during the response to epinephrine can be taken as evidence for the location of adrenergic receptors in the cell membranes is based on the assumption that electrical changes in the membrane control the changes in the contractile activity of smooth muscle cells. The very interesting recent note of Evans and Schild (21) raises some doubts about this basic assumption. They report that several different smooth muscle preparations, completely depolarized in "potassium sulfate Ringer," still contract when exposed to agents such as acetylcholine, histamine, 5-hydroxytryptamine and oxytocin. Although no results were reported on the action of epinephrine on smooth muscles which are stimulated by this agent, it seems likely that if acetylcholine can cause contraction of smooth muscle without electrical changes in the membrane, then epinephrine can do likewise. More detailed reports on the ability of drugs to cause contraction in completely depolarized smooth muscle are awaited with interest.

3′,5′-AMP, which is a co-factor for the activation (64). Rall and Sutherland (50, 51) leave the question open as to whether epinephrine accelerates the rate of synthesis or inhibits the rate of hydrolysis of the cyclic 3′,5′-AMP. Their finding that epinephrine can enhance the net formation of this activating substance in suspensions of cell-free particulate fractions from liver, heart and skeletal muscle provides even stronger evidence for an intracellular location of the adrenergic receptors for glycogenolysis. However, there is still some possibility that their particulate fractions contain fragments of cell membranes, and that the receptors are in these fragments.

Some investigators have proposed that the activation of glycogenolysis by epinephrine and related catecholamines may even account for some of the mechanical and electrical changes produced by these agents in smooth muscle and heart. Mohme-Lundholm (44, 45) has contended on the basis of pharmacological and chemical findings that the relaxation of smooth muscle by catecholamines is due to an increase in intracellular lactic acid resulting from the activation of glycogenolysis. Indirect evidence against this hypothesis, based on studies with smooth muscle largely depleted of glycogen or in the presence of the glycolytic inhibitor, glyceraldehyde, was presented by me in a previous review (24). Bentley (7) has also presented such indirect evidence, and has, in addition, been unable to confirm Mohme-Lundholm's finding of an increase in lactic acid in intestinal strips upon relaxation with epinephrine. More recent work in our laboratory (29) has also shown that isoproterenol and epinephrine (after Dibenamine blockade) inhibit the contraction of isolated strips of rabbit stomach muscle without producing a detectable rise in lactic acid concentration. Thus for the time being, it appears unlikely that lactic acid formation as a result of activated glycogenolysis can account for the inhibitory action of catecholamines on smooth muscle.

Ellis (20) has proposed that the increase in hexosemonophosphate concentration resulting from the activation of glycogenolysis by epinephrine may somehow lead to the increase in contractile force of skeletal muscle obtained with epinephrine under certain experimental conditions. He has also speculated that increases in hexosemonophosphate produced by epinephrine and other catecholamines may play a role in the positive inotropic action of these agents on heart muscle (17). We look forward with interest to what Dr. Ellis may have to say on this subject at the present symposium. However, in view of the separate findings that certain ergot alkaloids can block the glycogenolytic action of epinephrine (18) but cannot block its positive inotropic action on heart (48), it seems highly unlikely that increased hexosemonophosphate can account for the positive inotropic action.

With respect to the changes in ionic and electrical characteristics of intestinal smooth muscle and cardiac muscle cells produced by epinephrine, Shanes (56, 57) has proposed that they may be due in part to the accumulation of organic phosphates. These products of glycogenolysis, because they are acids with indiffusible anions, might reduce potassium and phosphate leakage, and also contribute hydrogen ions to augment potassium ion uptake by exchange through

the membrane. Such ionic interactions, according to Shanes, might be expected to lead to a better sustained membrane potential and to hyperpolarization.

The various proposals cited above are all concerned with the possibility that the action of a catecholamine on a single type of receptor in an effector cell may lead to more than one type of measured response by the cell, namely, both increased glycogenolysis and a change in contractile or electrical properties. At present, the evidence supporting this possibility is not too convincing. Somewhat along the same lines, Burn and Robinson (12) at one time hypothesized that a single type of adrenergic receptor may mediate either contraction or relaxation of smooth muscle, depending on the particular experimental conditions. An attempt to verify this hypothesis was made in my laboratory in a series of carefully controlled experiments on spiral strips of rabbit aorta. No evidence favoring such an hypothesis was obtained, all the evidence being in favor of two distinct types of adrenergic receptors for contraction and relaxation, respectively (23). Indeed, the evidence not only indicated the existence of these two types of adrenergic receptors in the smooth muscle of the rabbit aorta, but also strongly supported the concept that single individual smooth muscle cells in this blood vessel contain both types (23, 24).

C. *On the mechanism of action of catecholamines at the receptor level*

It must be admitted immediately that we have no real understanding of the mechanism of action of catecholamines at the receptor level. In large part our ignorance stems from the fact that we are at present unaware of the real nature of adrenergic receptors. Many have speculated that these receptors may be constituents of the cell membrane which on interaction with a catecholamine somehow lead to changes in ionic fluxes through the membrane. For example, Burnstock (13) has proposed recently that the inhibition of spike activity in the guinea pig taenia coli by epinephrine may be due to the inhibition of the "carrier" for sodium and/or some other ion which is responsible for initiation of the action potential. Others have speculated that adrenergic receptors may be enzymes, for which catecholamines are either activators, inhibitors or even substrates. Certainly the work of Sutherland, Rall and co-workers, whose interesting latest findings are reported in THIS SYMPOSIUM, strongly suggests an enzymatic nature for the adrenergic receptors mediating the activation of glycogenolysis. The possibility that adrenergic receptors may be enzymes located in cell membranes has also been considered by many.

Relative to the possible enzymatic nature of adrenergic receptors, it is appropriate to introduce here the provocative recent findings of Brown *et al.* (9, 10). These investigators have reported that the amount of sympathetic transmitter (norepinephrine) in the venous outflow from cat spleen and from cat intestine after stimulation of sympathetic nerves to these organs is increased greatly after adrenergic blockade with Dibenzylene or Dibenamine. The explanation favored by them is that the sympathetic transmitter must combine with adrenergic receptors before it is inactivated, and that blockade of such receptors with the β-haloalkylamines used prevents inactivation of the transmitter and leads to its

greater output in the venous outflow. This explanation, however, is not the only one possible; and two others which should be considered are the following: a) that the β-haloalkylamines in some manner cause a much greater release of transmitter from the sympathetic nerve endings, and b) that these agents not only block certain adrenergic receptors but also, in addition, inhibit an enzyme (not monoamine oxidase) which is chiefly responsible for the inactivation of the transmitter. Indirect support for explanation a) comes from our finding that all of four β-haloalkylamines tested by us cause increases in force and rate of isolated guinea pig atria, as a result of their liberation of catecholamines within the atria (25, 30, 32). For the four agents tested, the order of potency is SY28 (N-α-naphthylmethyl-N-ethyl-β-bromoethylamine) > G-D131 (N-cyclohexylmethyl-N-ethyl-β-chloroethylamine) > Dibenzyline $\simeq$ Dibenamine. A release of catecholamines by β-haloalkylamines would also explain Schapiro's recent finding that Dibenzyline causes a marked reduction in the catecholamine content of heart, spleen and adrenal medulla of rats (55).

Indirect support of explanation b) to account for the results of Brown *et al.* (9, 10) comes from our finding that Dibenzyline treatment leads to a 5- to 10-fold potentiation of the positive inotropic action of epinephrine and norepinephrine on isolated guinea pig atria (30). Also G-D131 at a concentration of 10^{-6} markedly potentiates the contractile response of rabbit aortic strips to epinephrine and norepinephrine (26). (This β-haloalkylamine, unlike Dibenamine, Dibenzyline and SY28, does not block adrenergic receptors of aortic strips at the concentration noted.) These potentiating effects of β-haloalkylamines may well be due to inhibition of an enzyme which inactivates catecholamines. It will be of considerable interest to determine whether β-haloalkylamines, as well as older potentiating agents such as cocaine and ephedrine, inhibit the O-methylating enzyme of Axelrod *et al.* (4, 5, 40), which now appears to be the enzyme chiefly responsible for inactivation of catecholamines in the body.

D. *Concluding remarks*

As is evident in the first section of this review, the pharmacological classification of adrenergic receptors responsible for mediating various responses is not a settled matter. The only general agreement is that adrenergic receptors mediating increased contractile activity in various smooth muscles should be considered as one distinct class (alpha or Ac). At the risk of considerable criticism, I might propose the following modification of Ahlquist's classification: *alpha* receptors for contraction of smooth muscle; *beta* receptors for relaxation of smooth muscle other than that of intestine, and also for increases in rate and strength of cardiac contraction; *gamma* receptors for glycogenolysis; and *delta* receptors for inhibition of intestinal smooth muscle. It may be that *beta* receptors are adequate for adrenergic inhibition of tonic contractions (contractures?) in smooth muscle, but that a different type of receptor, termed *delta* here, is necessary for adrenergic inhibition of rhythmic contractions of smooth muscle associated with propagated action potentials, as in intestine.

Since the classification given above places receptors for glycogenolysis in a

separate class (*gamma*), it implies that stimulation of glycogenolysis is not responsible, either directly or indirectly, for the effects of catecholamines on the contractile activity of heart and smooth muscle. I must admit the attractiveness of the concept that some common primary metabolic action of catecholamines leads to all of the diverse final effects of these agents; but I feel that experimental evidence supporting such a concept is so slight, that it is preferable, for the present, to think in terms of several distinct types of adrenergic receptors. Where are these adrenergic receptors located in effector cells? What is their chemical and physical nature? And how do the reactions between them and catecholamines lead to the responses which we observe and measure? These are the very difficult questions which future research must attempt to answer.

REFERENCES

1. AHLQUIST, R. P.: A study of the adrenotropic receptors. Amer. J. Physiol. 153: 586–600, 1948.
2. AHLQUIST, R. P.: Adrenergic drugs. In: Pharmacology in medicine, 2nd ed., pp. 378–407, edited by V. A. Drill. McGraw-Hill, New York, 1958.
3. ARIËNS, E. J., VAN ROSSUM, J. M. AND SIMONIS, A. M.: Affinity, intrinsic activity and drug interactions. Pharmacol. Rev. 9: 218–236, 1957.
4. AXELROD, J. AND TOMCHICK, R.: Enzymatic O-methylation of epinephrine and other catechols. J. biol. Chem. 233: 702–705, 1958.
5. AXELROD, J., SENOH, S. AND WITKOP, B.: O-methylation of catechol amines *in vivo*. J. biol. Chem. 233: 697–701, 1958.
6. BACQ, Z. M. AND MONNIER, A. M.: Recherches sur la physiologie et la pharmacologie du système nerveux autonome. XV. Variations de la polarisation des muscles lisses sous l'influence du système nerveux autonome et de ses mimétiques. Arch. int. Physiol. 40: 467–484, 1935.
7. BENTLEY, G. A.: A study of the inhibitory action of adrenaline. I. Effects on the carbohydrate metabolism of isolated rabbit intestine. Aust. J. exp. Biol. med. Sci. 34: 485–494, 1956.
8. BOZLER, E.: An analysis of the excitatory and inhibitory effects of sympathetic nerve impulses and adrenaline on visceral smooth muscle. Amer. J. Physiol. 130: 627–634, 1940.
9. BROWN, G. L. AND GILLESPIE, J. S.: The output of sympathetic transmitter from the spleen of the cat. J. Physiol. 138: 81–102, 1957.
10. BROWN, G. L., DAVIES, B. N. AND GILLESPIE, J. S.: The release of chemical transmitter from the sympathetic nerves of the intestine of the cat. J. Physiol. 143: 41–54, 1958.
11. BÜLBRING, E.: Changes in configuration of spontaneously discharged spike potentials from smooth muscle of the guinea-pig's taenia coli. The effect of electronic currents and of adrenaline, acetylcholine and histamine. J. Physiol. 135: 412–425, 1957.
12. BURN, J. H. AND ROBINSON, J.: Reversal of vascular response to acetylcholine and adrenaline. Brit. J. Pharmacol. 6: 110–119, 1951.
13. BURNSTOCK, G.: The action of adrenaline on excitability and membrane potential in the taenia coli of the guinea-pig and the effect of DNP on this action and on the action of acetylcholine. J. Physiol. 143: 183–194, 1958.
14. DALE, H. H.: On some physiological actions of ergot. J. Physiol. 34: 163–206, 1906.
15. DRESEL, P. E.: Some cardiac effects of 1-(3',4'-dichlorophenyl)-2-isopropylamino ethanol (Lilly 20522), an agent blocking adrenergic inhibitory responses. Abstract of paper presented at Fall Meeting of Amer. Soc. Pharmacol. and Exper. Therap., Ann Arbor, Mich., 1958.
16. ECCLES, J. C. AND MAGLADERY, J. W.: Rhythmic responses of smooth muscle. Amer. J. Physiol. 90: 68–99, 1937.
17. ELLIS, S.: Increased hexosemonophosphate, a common factor in muscular contraction potentiated by treppe, a short tetanus, epinephrine, or insulin. J. Pharmacol. 113: 17–18, 1955.
18. ELLIS, S.: The metabolic effects of epinephrine and related amines. Pharmacol. Rev. 8: 485–562, 1956.
19. ELLIS, S., ANDERSON, H. L., JR. AND COLLINS, M. C.: Pharmacologic differentiation between epinephrine- and HGF-hyperglycemias: application in analysis of cobalt-hyperglycemia. Proc. Soc. exp. Biol., N. Y. 84: 383–386, 1953.
20. ELLIS, S., DAVIS, A. H. AND ANDERSON, H. L., JR.: Effects of epinephrine and related amines on contraction and glycogenolysis of the rat's diaphragm. J. Pharmacol. 115: 120–125, 1955.
21. EVANS, D. H. L. AND SCHILD, H. O.: Mechanism of contraction of smooth muscle by drugs. Nature, Lond. 180: 341–342, 1957.
22. FLECKENSTEIN, A. AND BASS, H.: Zum Mechanismus der Wirkungsverstärkung und Wirkungsabschwächung sympathomimetischer Amine durch Cocain und andere Pharmaka. Arch. exp. Path. Pharmak. 220: 143–156, 1953.
23. FURCHGOTT, R. F.: Dibenamine blockade in strips of rabbit aorta and its use in differentiating receptors. J. Pharmacol. 111: 265–284. 1954.
24. FURCHGOTT, R. F.: The pharmacology of vascular smooth muscle. Pharmacol. Rev. 7: 183–265, 1955.

25. Furchgott, R. F.: In: Shock and circulatory homeostasis, Trans. 5th Conf. pp. 302–303. Josiah Macy, Jr. Foundation, New York, 1955.
26. Furchgott, R. F.: Unpublished.
27. Furchgott, R. F. and Bhadrakom, S.: Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. J. Pharmacol. 108: 129–143, 1953.
28. Furchgott, R. F. and Ehrreich, S.: Unpublished.
29. Furchgott, R. F. and Kleinman, L.: Unpublished.
30. Furchgott, R. F. and Roseman, M.: Unpublished.
31. Furchgott, R. F., de Gubareff, T. and Grossman, A.: Release of autonomic mediators in cardiac tissue by supra-threshold stimulation. (In press, 1959.)
32. Furchgott, R. F., de Gubareff, T. and Kelch, H.: Unpublished.
33. Harvey, S. C. and Nickerson, M.: Adrenergic inhibitory function in the rabbit: epinephrine reversal and isopropylnorepinephrine vasodepression. J. Pharmacol. 108: 281–291, 1953.
34. Harvey, S. C., Wang, C. Y. and Nickerson, M.: Blockade of epinephrine-induced hyperglycemia. J. Pharmacol. 104: 363–376, 1952.
35. Hawkins, D. F.: Action of veratramine on isolated guinea-pig sinus-auricle preparations. Abstract of paper presented at Fall Meeting of Amer. Soc. Pharmacol. and Exper. Therap., Ann Arbor, Mich., 1958.
36. Hutter, O. F. and Trautwein, W.: Vagal and sympathetic effects on the pacemaker fibers in the sinus venosus of the heart. J. gen. Physiol. 39: 715–733, 1956.
36a. Innes, I. R. and Krayer, O.: Studies on veratrum alkaloids. XXVII. The negative chronotropic action of veratramine and reserpine in the heart depleted of catecholamines. J. Pharmacol. 124: 245–251, 1958.
37. Krayer, O.: Studies on veratrum alkaloids. VIII. Veratramine, an antagonist to the cardioaccelerator action of epinephrine. J. Pharmacol. 96: 422–437, 1949.
38. Krayer, O.: Studies on veratrum alkaloids. IX. The inhibition by veratrosine of the cardioaccelerator action of epinephrine and of norepinephrine. J. Pharmacol. 97: 256–265, 1949.
39. Krayer, O. and van Maanen, E. F.: Studies on veratrum alkaloids. X. The inhibition by veratramine of the positive chronotropic effect of accelerans stimulation and of norepinephrine. J. Pharmacol. 97: 301–307, 1949.
40. LaBrosse, E. H., Axelrod, J. and Kety, S. S.: O-methylation, the principal route of metabolism of epinephrine in man. Science 128: 593–594, 1958.
41. Lands, A. M.: The pharmacological activity of epinephrine and related dihydroxyphenylethylalkylamines. Pharmacol. Rev. 1: 279–309, 1949.
42. Lands, A. M.: Sympathetic receptor action. Amer. J. Physiol. 169: 11–21, 1952.
43. Lockett, M. F.: The transmitter released by stimulation of the bronchial sympathetic nerves of cats. Brit. J. Pharmacol. 12: 86–96, 1957.
44. Mohme-Lundholm, E.: The mechanism of the relaxing effect of adrenaline on smooth muscle. Acta physiol. scand. 29: suppl. 108, 1–63, 1953.
45. Mohme-Lundholm, E.: Effect of adrenaline, noradrenaline, isopropylnoradrenaline and ephedrine on tone and lactic acid formation in bovine tracheal muscle. Acta physiol. scand. 37: 1–4, 1956.
46. Moran, N. C. and Perkins, M. E.: Blockade of adrenergic stimulation of the heart by 1-(3′,4′-dichlorophenyl)-2-isopropylaminoethanol hydrochloride, the chloro-analogue of isoproterenol. J. Pharmacol. 122: 55A. 1958.
47. Morison, R. S.: The effects of adrenaline and of nerve stimulation on the mechanical and electric responses of uterine muscle. Amer. J. Physiol. 128: 372–381, 1939.
48. Nickerson, M.: The pharmacology of adrenergic blockade. Pharmacol. Rev. 1: 27–101, 1949.
49. Powell, C. E. and Slater, I. H.: Blocking of inhibitory adrenergic receptors by a dichloro analog of isoproterenol. J. Pharmacol. 122: 480–488, 1958.
50. Rall, T. W. and Sutherland, E. W.: Formation of a cyclic adenine ribonucleotide by tissue particles. J. biol. Chem. 232: 1065–1076, 1958.
51. Rall, T. W., Sutherland, E. W. and Berthet, J.: The relationship of epinephrine and glucagon to liver phosphorylase. IV. Effect of epinephrine and glucagon on the reactivation of phosphorylase in liver homogenates. J. biol. Chem. 224: 463–475, 1957.
52. Rall, T. W., Sutherland, E. W. and Wosilait, W. D.: The relationship of epinephrine and glucagon to liver phosphorylase. III. Reactivation of liver phosphorylase in slices and in extracts. J. biol. Chem. 218: 483–495, 1956.
53. Rosenblueth, A., Davis, H. and Rempel, B.: The physiological significance of the electrical responses of smooth muscle. Amer. J. Physiol. 116: 387–407, 1936.
54. Rothlin, E., Konzett, H. and Cerletti, A.: The antagonism of ergot alkaloids towards the inhibitory response of the isolated rabbit intestine to epinephrine and norepinephrine. J. Pharmacol. 112: 185–190, 1954.
55. Schapiro, S.: Effect of a catechol amine blocking agent (dibenzyline) on organ content and urine excretion of noradrenaline and adrenaline. Acta physiol. scand. 42: 371–375, 1958.
56. Shanes, A. M.: Electrochemical aspects of physiological and pharmacological action in excitable cells. Part I: The resting cell and its alteration by extrinsic factors. Pharmacol. Rev. 10: 59–164, 1958.
57. Shanes, A. M.: Electrochemical aspects of physiological and pharmacological action in excitable cells. Part II: The action potential and excitation. Pharmacol. Rev. 10: 165–273, 1958.
58. Sleator, W. and de Gubareff, T.: Personal communication.
59. Sleator, W., Furchgott, R. F. and de Gubareff, T.: Unpublished.
60. Stephenson, R. P.: A modification of receptor theory. Brit. J. Pharmacol. 11: 379–393, 1956.
61. Sutherland, E. W.: The effect of epinephrine and the hyperglycemic factor on liver and muscle metabolism *in vitro*. In: Phosphorus metabolism, vol. II, pp. 577–593, edited by W. D. McElroy and B. Glass. Johns Hopkins Press, Baltimore, 1952.

62. SUTHERLAND, E. W. AND CORI, C. F.: Influence of insulin preparations on glycogenolysis in liver slices. J. biol. Chem. 172: 737-750, 1948.
63. SUTHERLAND, E. W. AND CORI, C. F.: Effect of hyperglycemic-glycogenolytic factor and epinephrine on liver phosphorylase. J. biol. Chem. 188: 531-543, 1951.
64. SUTHERLAND, E. W. AND RALL, T. W.: Fractionation and characterization of a cyclic adenine ribonucleotide formed by tissue particles. J. biol. Chem. 232: 1077-1091, 1958.
65. WEBB, J. L. AND HOLLANDER, P. B.: The action of acetylcholine and epinephrine on the cellular membrane potentials and contractility of rat atrium. Circulation Res. 4: 332-336, 1956.
66. WEST, T. C., FALK, G. AND CERVONI, P.: Drug alteration of transmembrane potentials in atrial pacemaker cells. J. Pharmacol. 117: 245-252, 1956.
67. WOSILAIT, W. D. AND SUTHERLAND, E. W.: The relationship of epinephrine and glucagon to liver phosphorylase. II. Enzymatic inactivation of liver phosphorylase. J. biol. Chem. 218: 469-481, 1956.

DISCUSSION

THE RECEPTORS FOR EPINEPHRINE AND NOREPINEPHRINE

R. P. AHLQUIST

Medical College of Georgia, Augusta, Georgia

Dr. Furchgott has described several theories regarding the general nature of the adrenergic receptors. Most of these theories have been developed from observations of differential effector responses to one or more adrenergic substances, administered exogenously or caused to appear endogenously. These methods are limited primarily by the uncertainty of the exact relationship between the observed gross response and the effector response at the cellular level.

It might be of interest to present briefly the unpublished observations that led to the development of one of the current theories of adrenergic receptors. In a search for a substance that would effectively prevent the spasmogenic action of vasopressin on the myometrium, we studied several compounds related chemically to epinephrine. Although none was found suitable, some were noted to have effects that seemed contradictory to our naive ideas of the general relationship between chemical structure and adrenergic action. Phenylephrine was found to relax effectively the ileum, both intact and isolated; dioxyephedrine (*alpha*-methylepinephrine), an active depressor amine, was found to have relatively little activity as far as gut relaxation was concerned; and isoproterenol could induce contraction of the isolated rabbit myometrium. We were also surprised that racemic arterenol was less effective as a vasoconstrictor than racemic epinephrine. A systematic, comparative study of other adrenergic drugs suggested the following relatively simple postulate.

Consider a series of amines closely related to epinephrine and call them compounds A, B, C and D. If in this series the order of relative activity is the same on all structures having adrenergic receptors (for example, $A > B > C > D$ on the smooth muscle of blood vessels, the gut, uterus, nictitating membrane, etc.), then the differences in activity could be due entirely to the differences in chemical structure. If, however, the order of activity varies from structure to structure (for example, $A > B > C > D$ on blood vessels; $D > C > B > A$ on gut; $C > D > A > B$ on uterus), then these variations in relative activity must be due in part to actual differences in the receptors involved.

As we later published, only two orders of relative activity for the common catecholamines were found if the adrenergic effector responses were considered in the broadest sense and if some apparent species variations were disregarded. Another fundamental assumption had to be made: that all of the catecholethanolamines acted on the receptors in a manner at least qualitatively similar to epinephrine.

If it is true that the differences in effector response to various catecholamines are due to differences in receptors, then the simplest theory suggests that there are only two kinds