ADRENERGIC RECEPTORS 6536

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The concept of the adrenergic receptor, a specific cellular constituent with which the liberated adrenergic neurotransmitter or its exogenous analogs interact to initiate the physiological responses, is in principle little different from that applied to other neurotransmitters and hormones. General discussions of such receptors are given in a number of recent articles (1–7).

Included among many notable contributors to the development of the adrenergic receptor concept are Elliott (8) who first proposed epinephrine (E) as the neurotransmitter, Dale (9, 10) who revealed the excitatory and inhibitory components of E and sympathetic stimulation and with whom Barger (11) made the first significant structure-activity analysis, von Euler (12, 13), whose patient investigations revealed norepinephrine (NE) to be the dominant transmitter, the many workers (14) who have demonstrated a catecholamine (CA) release mechanism activated in varying degrees by many sympathomimetic amines, and Ahlquist (15) who divided adrenergic receptors into two distinct classes (α and β). This classification, originally based on the existence of two distinct orders of activity of agonists and subsequently on the existence of selective antagonists, is too well established (16) to warrant further discussion here. Yet, as recognized by Ahlquist (15) and emphasized recently by Axelsson (17) the α/β classification provides essentially a structural description of the ability of the receptor macromolecules to discriminate adrenergic ligands and does not correspond uniquely to a functional classification into excitatory and inhibitory responses.

In this review, which will be confined very largely to adrenergic receptors in smooth muscle, there is legitimate reason to attribute excitatory and inhibitory responses to increases and decreases respectively in the level of intracellular Ca^{++} ($[Ca^{++}_{INT}]$). Hence, the transduction process at the adrenergic receptor may be written,

$$L + Rec \rightleftharpoons L\text{---}Rec \xrightarrow[\text{steps}]{\text{intermediate}} [Ca^{++}_{INT}] \uparrow\downarrow \qquad 1.$$

Three basic questions, which remain to be answered satisfactorily, arise from this equation: What is the topography, localization, and constitution of the receptors? What is the source(s) of Ca^{++} supply (or removal) to the contractile machinery? What is the nature of the linkage or linkages between the initial ligand-receptor interaction and the Ca^{++} mobilization/immobilization process? All three questions must be answered to achieve a real understanding of adrenergic receptor structure and function.

Receptor Topography and Constitution

Despite potential ambiguities of interpretation, not likely to be resolved until isolated receptor macromolecules are available, structure-activity relationships are valuable in probing receptor topography. Recent reviews of adrenergic ligands are available (16, 18). Optimum direct agonist activity remains associated with the catecholamine structure, comparatively few structural deviations being permitted. A major distinction between α- and β-receptors lies in the tolerance of the latter for nonpolar N-substituents and in the possible nonessential character of the ammonium function for β-agonist activity (19, 20). The finding that the sulfonamido group can substitute effectively for the phenolic group (21) and that agonist activity resides only in the *m*-sulfonamidophenylethanolamines (3-RSO_2NH, 4-$HOC_6H_3CHOHCH_2NHR'$) with the *p*-derivatives exhibiting antagonism suggests that binding of the sulfonamido group may serve to orient the ligand at the receptor. A similar orientation effect may occur with the saligenin analogs (3-$HOCH_2$, 4-$HOC_6H_3CHOHCH_2NHR$) (22, 23). The R-stereoselectivity of agonist interaction at both α- and β-receptors has received considerable attention (24, 25): stereoselectivity appears greater at the β-receptors. Agents lacking the chiral center [1-(3,4,5-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, I, (26)] may owe their high β activity to restricted conformational flexibility and compensating binding by the N-substituent. Similar absolute stereoselectivity is exhibited by β-antagonists (21, 25, 27) as might be anticipated from their generally close structural similarity to the agonists (21, 28). In marked contrast, there is little structural resemblance between α-agonists and α-antagonists (21, 28) which may indicate nonidentical binding sites.

The structural delineation of two classes of adrenergic receptors seems clear; however, several classes of observations reviewed by Furchgott (29) and Brittain et al (23) and based largely on relative agonist and antagonist activities in series of tissues suggest the existence of subdivisions of these major classes. From these studies it is not evident that the subdivisions indicated by agonists and antagonists are always identical. Discrepancies may arise because: agonists and antagonists probe different areas of receptors; differing experimental conditions, particularly with regard to control of uptake, release, metabolic, and nonreceptor binding parameters (29, 30) have been employed; diffusion and transport differences related to the physicochemical properties of the ligands have not been considered; in addition the

heterogeneous distribution of receptors represents a complication only recently recognized (31, 32). Patil (33–36) has employed the isomer ratios of agonists and antagonists in appropriately treated tissues in an attempt to circumvent these difficulties. The (−) NE/(+) NE ratio indicated identity of six α-receptor preparations, but β-receptors fell into three distinct classes, (*a*) rat atria, (*b*) rabbit atria, guinea pig atria and trachea, and rabbit aorta, and (*c*) bovine iris sphincter.

The concept of distinct α- and β-receptors is, in part, based upon the selective actions of antagonists. However, cross interaction does occur as judged by the abilities of β-antagonists to inhibit (competitively and noncompetitively) α-receptor mediated events (37, 38) and to protect against inactivation by irreversible α-antagonists (39, 40). In part, such findings may be due to interactions of β-antagonists at the α-receptor proper (37, 40) and it is relevant to note that the binding requirements of some α-antagonists ($ArCHBrCH_2NMe_2$) and β-antagonists ($ArCHOHCH_2NHPr^i$) as judged by regression analysis are similar (41, 42). However, much of this mutual interaction appears related to the ability of many β-antagonists to interact at Ca^{++} binding sites; a number of the more lipophilic β-antagonists have pronounced local anesthetic activities (16, 37, 38, 40, 43, 44) and an ability to interfere with catecholamine-induced Ca^{++} transport into lipids (45, 46). Thus, the extent of noncompetitive inhibition and degree of protection exerted against irreversible α-antagonists (Dibenamine, etc.) appears determined by the local anesthetic activity of the β-antagonist (38, 40). Such findings constitute suggestive evidence of an integral role of Ca^{++} in ligand interaction at adrenergic receptors, a point that will be reemphasized later.

Attempts to probe more directly the structure of receptors have not been particularly successful. The use of irreversible antagonists as labeling species (47, 48) still appears to suffer from chemical nonspecificity (49). However, de Plazas & De Robertis (personal communication) have isolated a proteolipid from bovine spleen capsule to which norepinephrine binds with high affinity and is displaced by adrenergic antagonists. Interestingly, their estimate of receptor sites in this material compares very favorably with values obtained by entirely different techniques in the rat seminal vesicle and rabbit aorta (47, 48). An attempt has been made (50), using radioautography with ^3H-phenoxybenzamine, to localize the α-receptor of pancreatic arteriolar muscle. A widespread diffusion of the label over cytoplasm and nuclei was found which was diminished by protection with norepinephrine without, however, any change in the cellular distribution pattern. Interestingly, no concentration of label was found on cell membranes.

Calcium: The Sites of Origin

The final step of the transduction Equation 1 raises the question of the origin(s) of the Ca^{++} supplied to or removed from the contractile ma-

chinery during the mechanical changes initiated by the adrenergic neurotransmitter.

This question, applied generally to neurotransmitters, has been the subject of much work and is discussed in recent reviews (51–59). In principle, three sources are recognized: bound intracellular Ca^{++}, free extracellular Ca^{++}, and extracellular membrane-bound Ca^{++}. A priori calculations taking into account the sizes of smooth muscle cells (60), presumed levels of Ca^{++}_{INT} (54), membrane areas and anionic site concentrations (53, 57), and the amounts of sacroplasmic reticulum and actomyosin present do not permit a clear distinction to be made between these sources, and the mechanical properties of most smooth muscles are such that any of these sources could be appropriate. In any event, the variability in properties of smooth muscle is such that any conclusions drawn for one system should not be overhastily extended to other systems.

The specific situation with adrenergically mediated processes is still complex. Subsequent to the early work of Cow (61) on sheep arteries a number of reports show that CA sensitivity may be retained for a substantial time in the absence of Ca^{++}_{EXT} while K^+ sensitivity is lost far more rapidly in many tissues (51, 53, 55, 59, 62–68). An obvious, although not exclusive (51, 52, 69, 70), conclusion is that intracellular and extracellular sources of Ca^{++} are utilized by CAs and K^+ respectively. Analysis of $^{45}Ca^{++}$ desaturation of rabbit aorta reveals a biphasic process (71) and the slower component ($t_{1/2} = 108$ min) may represent that utilized by NE (67). Not all smooth muscles behave thus; the rat vas deferens loses sensitivity very rapidly to both K^+ and NE, and the dependence of these two activators on $[Ca^{++}_{EXT}]$ is very similar (73), suggesting utilization of Ca^{++}_{EXT}. Nevertheless, this is not by the same mechanism, for in the vas deferens (72), as in the rabbit aorta (74), SKF525A selectively antagonizes K^+-induced responses. There is evidence also that some tissues, among them the rabbit aorta, may utilize two sources of Ca^{++}; the fast and slow components of the NE-induced contraction of this tissue show differential sensitivity to Ca^{++}_{EXT}, the fast component being depressed and the slow component enhanced (62). It is suggested (62, 75) that the fast compound represents a membrane-induced (stabilized by high Ca^{++}_{EXT}) release of Ca^{++}_{INT} and the slow process a mobilization of Ca^{++}_{EXT}, perhaps through a Na^{+}_{INT}-Ca^{++}_{EXT} exchange pump (75). Studies on Ca^{++} fluxes, discussed in a number of papers and reviews (51, 53, 54, 56, 58, 76, 77) have not been very helpful in defining with precision the locus of drug-induced Ca^{++} movements largely because of a general lack of understanding of the structural parameters of smooth muscle. It is being increasingly realized that the Ca^{++} mobilized by catecholamine (and other neurotransmitters) action may represent only a very small fraction of that observed (58, 78, 79). However, an interesting attempt to define this fraction has been made (80, 81). La^{+++} blocks Ca^{++} fluxes across artificial membranes (82) and van Breeman has argued that it may be utilized to separate cellular from extracellular Ca^{++} exchange. In accord with this, La^{+++} inhibits only the

slow component of the NE-induced response and the fast component of the K^+-induced response of rabbit aorta, processes judged by other criteria also (62, 75) to be dependent upon Ca^{++}_{EXT}. Furthermore, comparison of tension developed by Na^+, Li^+ and K^+ showed no correlation with $^{45}Ca^{++}$ influx. However, a very satisfactory correlation was found when the extracellular Ca^{++} component was removed by La^{+++}. This method appears to offer potential for the isolation of Ca^{++} components involved in excitation-contraction coupling.

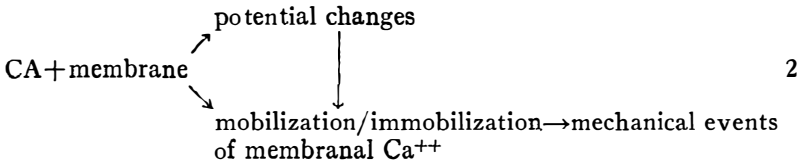
The Receptor-Ca^{++} Linkage

How the initial CA-receptor complex formation is coupled to the final step of Ca^{++} mobilization/immobilization in Equation 1 remains to be solved (51, 58, 83). Several distinct coupling mechanisms may exist, and progress is being made in their elucidation, but the molecular mechanisms are still obscure. Examination of smooth muscle systems reveals that a variety of potential, permeability, and biochemical changes accompany the actions of catecholamines. What has to be determined for each system is which of the observed changes are merely epiphenomena (69) and only incidental to the main transduction pathway. However, in establishing this, it cannot be ignored that necessary changes in experimental conditions may cause changes in coupling mechanisms.

One obvious coupling mechanism is that of control of Ca^{++} availability by regulation of membrane potential. The obvious and well documented (51, 52, 60, 77, 84–86) structural and functional heterogeneity of smooth muscle, particularly with regard to the presence or absence of spike potentials and graded depolarizations, and the question of coincidence of such changes with mechanical events, suggests that several regulatory mechanisms may exist. Since the resting membrane potential (RMP) of smooth muscle is low because of high Na^+ permeability, decreased Na^+ conductance, increased Na^+ pumping, or increased K^+ conductance will lead to hyperpolarization. Excitation, either by spike or graded potential changes, can arise by Na^+ spikes, decreased K^+ permeability, decreased Na^+ pumping or Ca^{++} spikes. The latter event has the attractive feature that it permits a rather direct excitation-contraction (E-C) coupling. Finally, inhibitory activity may also arise in rhythmically active tissues through suppression of generator potentials. Examples of most, if not all, of these processes have been reported although the electrogenic pump mechanisms in particular have not been established beyond reproach (62, 70, 77, 85–96). However, since all of these processes are categorized, on the basis of structure-activity relationships, as either α- or β-receptor events, it appears highly probable that they possess a common component of membrane function. A Ca^{++} binding site specifically linked to the catecholamine recognition site of the receptor appears a likely common component whereby increased or decreased binding of Ca^{++} at this site mediates inhibitory or excitatory responses respectively. Expressed in this general form, the hypothesis is entirely consistent with the well known stabilizing and labilizing effects of Ca^{++} on membrane function (83). A

similar controlling role has been advanced for membrane bound Ca^{++} in nerve excitation (97, 98). Essentially this hypothesis has been advanced explicitly by several workers (20, 51, 69, 70, 91, 99–101). For inhibitory processes the hyperpolarizing effect of E in rat uterus (101) is lost in the absence of Ca^{++} and in taenia coli, where both α- and β-receptors mediate mechanical relaxation [the α-effect through increased K^+ permeability (96, 98, 99) and the β-effect through a reduction of the generator potential (89, 90)] the effects of E are potentiated by increased Ca^{++}, reduced in lowered Ca^{++} and abolished by Mn^{++} and Ba^{++}.

However, the role of potential changes as a necessary step in the CA initiated transduction pathway should not be overemphasized. It is now quite well established in many tissues that the link between potential and mechanical events is not obligatory (51, 55, 58, 59, 69, 83, 86, 102). CAs can produce mechanical changes in polarized tissues with dissociation or absence of potential changes (51, 55, 69, 70, 103–107), they can produce mechanical changes in K^+-depolarized tissues, and the pharmacological criteria of receptor activity do not appear sensibly different in polarized and depolarized tissues (51, 87, 108–110). Furthermore, CA-induced changes in K^+ depolarized tissues are greater than and additive upon Ca^{++} induced contractures, and the characteristic differences in maximum response with different agonists occur in polarized and depolarized tissues (51, 69, 70). Hence, in many instances, the characteristic features of adrenergic (and other hormones) ligand interactions are transduced by mechanisms that can be independent of changes in membrane potential. A fundamental role of membrane-bound Ca^{++} seems probable (70):



2.

It is of interest that some years ago Belleau (111) organized a form of the α-receptor topography that featured a key role of phospholipid associated Ca^{++} in the activation pathway.

In depolarized rat uterus the relaxing effect of isopropylnorepinephrine (ISO) is antagonized by the Ca^{++} chelators, EDTA and EGTA (109) and in depolarized taenia coli ISO is effective in reducing Ca^{++} contractures, (87), perhaps preventing Ca^{++} entry by increasing membrane bound Ca^{++}. Documentation of labilization of membrane bound Ca^{++} in α-excitatory responses appears less readily available; in any event, the effects of $[Ca^{++}_{EXT}]$ variation will tend to ambiguity of interpretation because of simultaneous effects on membrane potential and on the Ca^{++} availability for the contractile process, in addition to altered binding at the proposed CA-associated site. However, elevated $[Ca^{++}_{EXT}]$ has been shown to depress α-responses (61, 62, 112) and

this may involve both general and specific membrane stabilization. Similarly, the findings that local anesthetics, diazoxide, papaverine, and other spasmolytics serve as noncompetitive antagonists of such excitatory processes (102, 113–116) are not uniquely interpretable since they also act as general membrane stabilizers.

Nevertheless, scattered results do suggest that known adrenergic agents may involve themselves quite specifically at sites of Ca^{++} function (83). In particular, 2-halogenoethylamines (2-HEs) (Dibenamine, etc.) inhibit K^+-induced contractions and associated Ca^{++} movements, although at concentrations significantly higher than those required for α-antagonism (117, 118). However, more specific effects are found with Dibenamine ($\sim 10^{-5}$M) inhibition of Ca^{++} contractures of vascular smooth muscle (86), and a definite association of 2-HEs with a specific Ca^{++} site has been described in the rat vas deferens and rabbit aorta (72, 119). This may bear on the fact that 2-HEs inactivate excitatory processes initiated by a variety of ligands, including acetylcholine, histamine, and 5-hydroxytryptamine (119). The protection of aortic α-receptors by E against Dibenamine activation requires Ca^{++} (120). E binding to rat liver plasma membranes is inhibited by propranolol and enhances Ca^{++} binding (121, 122). CAs promote uptake of Ca^{++} into lipid phases from aqueous solution and this is inhibited by β-antagonists (45, 46). In the presence of low concentrations of Ca^{++}, NE and ATP undergo phase separation as a complex from aqueous solutions (123).

Of related interest are the nonspecific smooth muscle supersensitivities (to CAs, acetylcholine, K^+) produced by reserpine and preganglionic denervation (124, 125) and which may relate to an increased tissue affinity for Ca^{++} (126, 127). Under supersensitive conditions Dibenamine is reported to be significantly less effective (128).

Reports have appeared that adrenergic ligands can facilitate and inhibit uptake of Ca^{++} into cardiac sarcoplasmic reticulum (129–131). There appears justifiable skepticism that these findings are related to cardiac E-C coupling (132, 133) but the possibility exists that they may be indicative of some affinity for adrenergic ligands better developed in smooth muscle membranes. In smooth muscle it may be that the poor development of the sarcoplasmic reticulum relative to cardiac and skeletal muscle delegates its function to the smooth muscle membrane (134).

A model can be envisaged for these CA-Ca^{++} interactions whereby the binding of CAs at the recognition sites characterizing the α- and β-receptors serves to modulate Ca^{++} binding directly at a specifically linked site. That Ca^{++} asymmetries across model membranes enhance electrical and mechanical instability may be relevant to this condition (135). However, intermediaries may be involved in this process and a likely candidate is cyclic 3′, 5′-adenosine monophosphate (c-AMP) famed for its ubiquitous involvement in many hormonal and regulatory processes including adrenergic events (136).

At the membrane potential level c-AMP and ISO hyperpolarize rat liver cells (137), a delayed process accompanied by increased K^+ efflux but pre-

ceded by an increased Ca^{++} efflux. In vascular smooth muscle (138) ISO or dibutyryl c-AMP, both in the presence of theophylline, produced significant hyperpolarization in low K^+ media. These findings suggest a role of c-AMP in β-mediated hyperpolarizations perhaps exerted at the inner membrane surface (145).

Relaxation of taenia coli (139) and rabbit colon (140) by E and ISO are associated with increases in c-AMP, but colon relaxation by phenylephrine (an α-stimulant) is associated with a decrease in c-AMP; CA-induced uterine relaxation (141, 142) is associated with increases in c-AMP blocked by propranolol but not by phentolamine. c-AMP and its more lipophilic less readily hydrolyzed dibutryl analog have direct effects on smooth muscles (143–148); in rabbit intestine relaxation these agents mimic the effects of phenylephrine (α) and ISO (β) respectively (14). Potentiation of adrenergic responses by methylxanthines, phosphodiesterase inhibitors, is also cited as evidence for c-AMP involvement (136, 141, 143, 147–151): the relaxant effects of papaverine (and other spasmolytics) have also been attributed to their activity as phosphodiesterase inhibitors (152–154). Increased c-AMP levels have been associated not only with α- and β-inhibitory effects, but also with α-excitatory effects in smooth muscle (143, 148, 149). Caution is necessary in interpretation, however, of many of these findings: adenosine and adenine nucleotides other than c-AMP have been known for many years to relax smooth muscle systems (145, 155) and ATP, or a related nucleotide, has been proposed as an intestinal inhibitory transmitter (156). The methylxanthine phosphodiesterase inhibitors are equally well known for their involvement at sites of Ca^{++} interaction and, as shown by Levy & Wilkenfeld (157), theophylline potentiates the inhibitory responses of rat uterus to NE and nitroglycerine; the latter, an agent considered to be a depressant devoid of activity on adenyl cyclase, also potentiated the response to NE.

Several arguments do support a link between c-AMP and Ca^{++} at both the membrane and intracellular levels of regulation (158, 159). For smooth muscle the evidence is meager: dibutyryl c-AMP is reported to increase intestinal permeability to Ca^{++} (160) and to inhibit Ca^{++}-induced contractions in rat uterus, and a presumed endogenous increase in c-AMP produced by a combination of ineffective concentrations of E and theophylline inhibits the Ca^{++}-dependent oxytocin contractions of uterus (150, 151, 161).

It seems quite clear that before one can write with authority on the chain of events involved in the transduction of CA-induced processes and on the molecular basis of such events, a great deal more information is needed on the roles and sites of action of Ca^{++} and c-AMP. May it be the pleasant task of the next reviewer of this topic to write with such authority.

ACKNOWLEDGMENTS

The preparation of this review was supported by a grant from the National Institutes of Health (NS-09573).

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