Receptor Binding Studies in Adrenergic Pharmacology

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Preface

The study of adrenergic receptors has been an explosive area of investigation in recent years. The advent of new radioligand binding techniques has provided a method for probing a number of questions regarding the structure, function, and regulation of adrenergic receptor sites. The goals of this monograph on adrenergic receptors are threefold:

1. The historical, theoretical, and pharmacological background of adrenergic binding studies is discussed. We feel that the theoretical (Chapter 4) and pharmacological (Chapter 2) discussion will be of particular value for the reader who is a "nonpharmacologist." Portions of the information in these chapters have been derived from the sources cited in the reference lists, while a significant part of this discussion has been developed especially for this monograph. We are hopeful that this synthesis is useful and we welcome any suggestions for improvement.

2. The methods which are currently being used for adrenergic binding studies are described in great detail (Chapters 5–7). An attempt has been made to include discussions of the pitfalls and shortcuts that we and others have found, but have not included in previously published formal manuscripts.

3. Examples of the information that can be derived from adrenergic binding studies are described (Chapters 6–10). We have attempted to review the results of many laboratories working in this area. However, we have chosen, for convenience, to illustrate many of the salient points with data from our own laboratory. In addition we have attempted to discuss the results of adrenergic receptor studies in the context of how they might be applicable to other hormone receptor systems.

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Contents

Chapter 1

Introduction

The catecholamines are remarkable biological agents. Despite their structural simplicity, these compounds induce responses as diverse as glycogenolysis, aggregation of platelets, contraction of the myometrium, increased contractility of the heart, and lipolysis in adipose tissue. For over 60 years investigators have attempted to elucidate the manner in which these compounds interact with responsive tissues. Out of research in this area has evolved the concept that catecholamines initiate biological responses by first interacting with discrete sites on the cell membrane. In 1906 Dale (1) discovered that ergot alkaloids inhibit some of the responses to catecholamines but do not inhibit others—an indication that more than one type of interaction of catecholamines with responsive tissues exists. Clark (2), in 1937, postulated that the actions of catecholamines, as well as other drugs, are mediated by the interaction with specific cell surface structures termed "receptors." The response is the proportional to the number of receptors occupied by the drug. The adrenergic receptor concept was extended by Ahlquist (3), who performed a study of the responses of a variety of tissues to a series of structurally different catecholamines. The results demonstrated that the responses could be divided into two distinct groups based on the order of potency of the agonists in eliciting the responses. Ahlquist concluded that there must be two types of catecholamine receptors, which he termed "alpha" and "beta."

In subsequent studies by other investigators, the receptor concept was further strengthened by the use of competitive antagonists that could selectively inhibit either alpha- or beta-adrenergic responses. This selective inhibition was presumed to be due to blockade or occlusion of the receptors by the antagonist, thus preventing access to the receptor by agonists. Further studies with stereoisomers revealed that the three-dimensional arrangement of atoms in the catecholamine molecule is a critical factor in determining biological potency. Thus it appeared that an exact "fit" of the catecholamine into a receptor site was required. Finally, detailed analyses of the quantitative relation between the concentration of drug and the degree of biological response demonstrated a close similarity to adsorption isotherms and to enzyme-substrate reaction curves, suggesting that the catecholamine molecule first binds to a receptor recognition site before eliciting a biological response in the cell.

Hence a large amount of information has indirectly suggested the ex-

istence of alpha- and beta-adrenergic receptors. Adrenergic receptors, like other hormone receptors, presumably have two functions in mediating the actions of their respective hormones. First, the receptor is responsible for selective recognition and binding of specific molecular structures, thereby functioning as a discriminator. Second, the receptor is responsible for initiating a sequence of steps that result in the physiological response to the catecholamine. In experiments antedating direct binding studies, information about the recognition function of receptors could only be inferred from measurements of the biological responses to adrenergic agents. Because of this limitation, conventional pharmacological studies have not provided a clear understanding of how receptors discriminate among biological molecules and how they transmit information to the interior of the cell.

Recently a new approach to the study of adrenergic receptors has been the use of radioactive adrenergic ligands[1] (radioligands) as probes to directly identify and study the receptor sites. By this technique the receptors can be quantitated, their specificity can be defined, the kinetics of their interactions with adrenergic ligands can be examined, their localization within the cell can be determined, and specific information about their role as transducers of information from the catecholamine to the cellular machinery can be deduced. In addition, alterations in the number or characteristics of receptors in various physiological or pathological states can be directly examined.

The purpose of this monograph is to describe the history, theoretical basis, and methodology of radioligand binding studies of the adrenergic receptors and to provide the reader with examples of how these techniques can be used to study a wide array of pharmacological, physiological, and biochemical problems. Within the last three years the increase in investigative efforts in this area has been explosive and has generated a large body of literature. Although we have attempted to update the reader on the main advances in this field, we have been unable to include an exhaustive discussion of the extant literature. Wherever possible we have provided experimental data to support the principles being discussed. Although many of the data presented come from our own laboratory, an attempt has been made to discuss this work in the context of data generated by other investigators. In addition, we have included discussions of how these studies of adrenergic receptors provide information that might be applicable to other hormone receptor systems. It seems clear that the radioligand binding approach to receptor investigation will continue to be a valuable tool in elucidating the structure and biological function of adrenergic as well as other hormone receptors.

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[1] The term ligand is used to mean "an atom, group of atoms, or a molecule that binds to a macromolecule," as defined in *Dictionary of Biochemistry* (John Wiley and Sons, New York, 1975) by J. Stenesh.

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Chapter 2

Pharmacology of Adrenergic Receptors

During the last three decades a vast amount of literature has accumulated on adrenergic pharmacology. The purpose of this chapter is to review the essential concepts in this area and to provide a framework for more detailed discussions in subsequent chapters of this monograph. First, the classification of adrenergic receptors within the autonomic nervous system is discussed. In a subsequent section, the pharmacological agents themselves are discussed and divided into groups according to their principal pharmacological effects.

CLASSIFICATION AND DEFINITION OF ADRENERGIC RECEPTORS

Historically the concept of alpha- and beta-adrenergic receptors arose out of attempts to subdivide the autonomic nervous system into its functional components. In this context, alpha- and beta-receptors are classes of end organ receptors at the termini of adrenergic neurons that are components of the sympathetic pathways of the autonomic nervous system. The definitions of these terms are considered in the following discussion. A classification scheme for the portions of the autonomic nervous system relevant to this discussion is shown in Fig. 2–1.

The autonomic nervous system is the part of the nervous system concerned with the regulation of visceral activities and is comprised of the nerve cells and fibers that are distributed to smooth muscle, cardiac muscle, and glands. The efferent autonomic nervous system is divided, on the basis of the anatomy of the outflow of nerves from the central nervous system, into two portions designated thoracolumbar (sympathetic) and craniosacral (parasympathetic). The term "sympathetic" is an anatomical term referring to the neural pathways that originate from neurons with their cell bodies in the thoracolumbar segments of the spinal cord. The parasympathetic pathways originate from neurons that have their cell bodies in the midbrain, the medulla, or the sacral portion of the spinal cord.

The sympathetic pathways consist of three major components—preganglionic neurons, postganglionic neurons, and responsive tissues. The preganglionic neurons, which originate in the spinal cord, synapse with the postganglionic neurons in the sympathetic trunk ganglia or in specialized collateral ganglia. The postganglionic neurons then terminate at nerve endings on the responsive organs. Most, but not all, postganglionic sympathetic

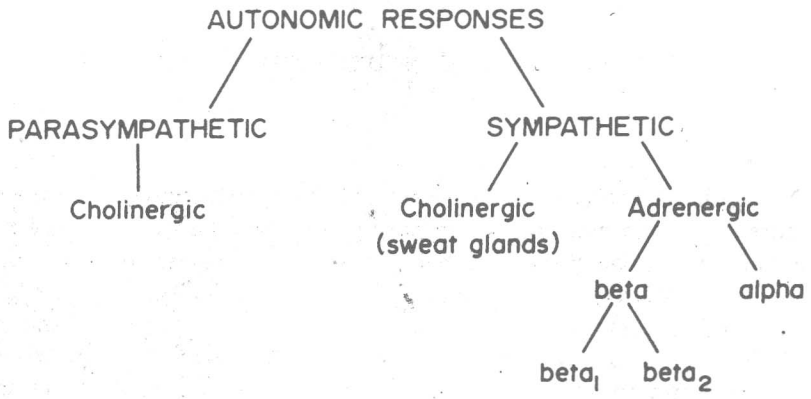

FIG. 2–1. Classification of autonomic responses. The division between parasympathetic and sympathetic responses is based on the anatomical location of the nerve cell bodies. Adrenergic and cholinergic responses are distinguished by the chemical nature of the neurotransmitter released at the end organ. Beta and alpha responses are distinguished by the pharmacological specificity of the responses.

fibers release catecholamines from their endings on the responsive organs and are designated as *adrenergic* neurons. Thus, the term "adrenergic" refers to the neurons that release a particular type of transmitter, whereas the term "sympathetic" is a more inclusive term that refers to autonomic neural pathways with a certain anatomical origin (thoracolumbar).

The end organ receptors for adrenergic transmitters have been termed "adrenergic receptors," and the responses elicited by stimulation of adrenergic nerves are termed "adrenergic responses." *In vivo,* these responses can be elicited by (a) catecholamine transmitters (especially norepinephrine) released at the nerve ending as a result of adrenergic nerve stimulation, (b) catecholamines, especially epinephrine, released into the circulation from the adrenal medulla, and (c) parenterally administered catecholamines or related adrenergic agents.

In vitro, adrenergic responses can be elicited by exogenous catecholamines in denervated or in innervated preparations, thereby indicating that the nerve ending *per se* is not required for the response. The specialized part of the effector cell through which adrenergic compounds act to evoke a characteristic response is defined as the adrenergic receptor.

It should be pointed out that, although historically adrenergic responses were defined in organs that have adrenergic innervation, it is now clear that there are responses in noninnervated tissues (e.g., circulating leukocytes) that are pharmacologically identical to the classic adrenergic responses and hence are mediated by adrenergic receptors.

In 1948, Raymond P. Ahlquist proposed that there are at least two types of adrenergic receptors (1). For his studies, which were originally designed to search for a specific uterine relaxant that might be effective in relieving dysmenorrhea, Ahlquist utilized five different catecholamines—norepineph-

rine, epinephrine (*dl* and *l* forms), methylnorepinephrine, methylepinephrine, and isoproterenol. Ahlquist then screened these compounds for their adrenergic effects on several organs and found that the organ responses could be placed into two distinct groups based on the order of potency of the compounds in eliciting the responses. For the first group of responses, the order of potency was epinephrine > norepinephrine > isoproterenol, whereas in the second group of responses, the order was isoproterenol > epinephrine ≥ norepinephrine. He designated these groups "alpha" and "beta," respectively. Subsequent work by many investigators demonstrated that all adrenergic responses appear to fall into one of these two general categories. The alpha-adrenergic responses to catecholamines include smooth muscle contraction and platelet aggregation (see Table 1). The beta-adrenergic responses include smooth muscle relaxation, the positive inotropic and chronotropic cardiac responses, and metabolic responses such as lipolysis.

In addition to their differential responsiveness to catecholamines, alpha- and beta-adrenergic responses can be distinguished by the specific antagonists that block the respective responses (Fig. 2–2). For example, alpha-adrenergic responses are blocked by compounds such as phentolamine, phenoxybenzamine, and dihydroergocryptine (an ergot alkaloid), whereas beta-adrenergic responses are antagonized by propranolol, practolol, and dihydroalprenolol. The main pharmacological features used to differentiate between alpha- and beta-adrenergic receptors are summarized in the schematic diagram of a smooth muscle cell in Fig. 2–2; the structures of some representative compounds are shown in Fig. 2–3. Alpha- and beta-adrenergic responses can also be distinguished by the biochemical events that accompany these re-

TABLE 1. *Some typical adrenergic responses*

Tissue	Alpha response	Beta response
Smooth muscle		
Uterus (rabbit)	Contraction	Relaxation
Pyloric sphincter	Contraction	Relaxation
Bronchial		Relaxation
Bladder (detrusor)		Relaxation
Bladder (trigone and sphincter)	Contraction	
Iris (radial muscle)	Contraction	
Ciliary muscle (lens)		Relaxation
Intestine	Decreased motility	Decreased motility
Arterial	Contraction	Relaxation
Adipose tissue		Lipolysis
Salivary glands	$K^+ + H_2O$ secretion	Amylase secretion
Lymphocytes		Inhibition of cytolysis
Cardiac muscle		
Contractility	Increase	Increase
Heart rate		Increase
Functional refractory period	Increased (ref. 20)	Decreased
Platelets	Aggregation	Inhibition of aggregation

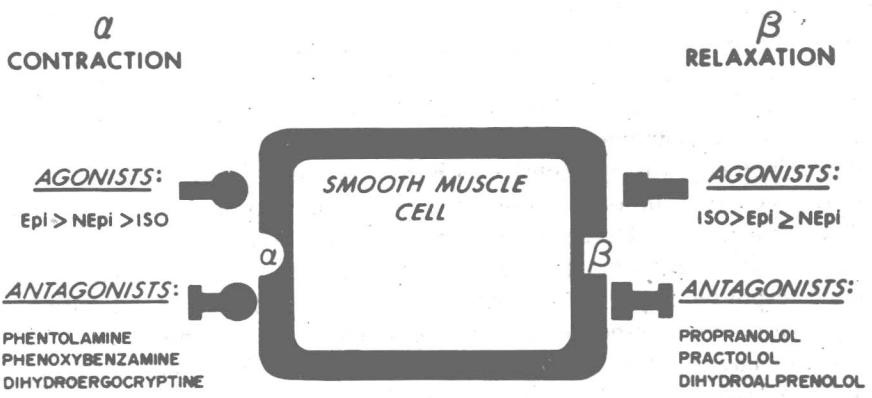

FIG. 2–2. The pharmacological differentiation of alpha- and beta-adrenergic responses. A schematic diagram of a typical smooth muscle cell is shown.

sponses. In most tissues beta-adrenergic responses are associated with an increase in the formation of cyclic AMP by the enzyme adenylate cyclase. This increase in cyclic AMP is thought to mediate the intracellular processes that cause the beta-adrenergic responses. By contrast, alpha-adrenergic stimu-

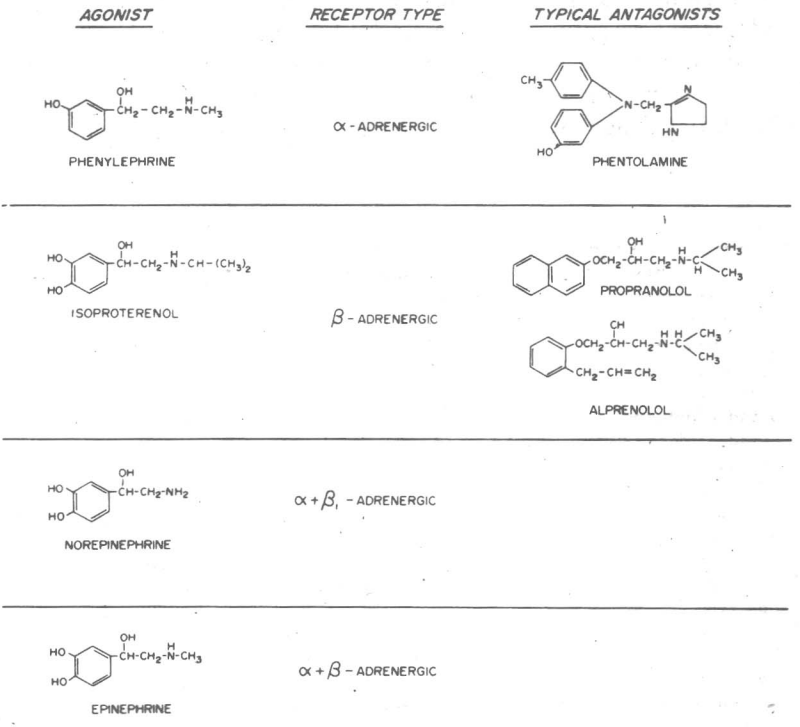

FIG. 2–3. Structures of some representative alpha- and beta-adrenergic compounds.

lation is in general associated with a decrease or no change in the intracellular level of cyclic AMP. The brain is exceptional in that alpha-adrenergic stimulation has been reported to cause an increase in cyclic AMP (3). Hence the two classes of adrenergic response appear to involve distinct biochemical mechanisms.

The group of beta-adrenergic responses can be further subdivided into at least two subgroups, as described by Lands (2). In the first group, $beta_1$, the catecholamines epinephrine and norepinephine are approximately equipotent in eliciting a response, whereas in the second group, $beta_2$, epinephrine is much more potent than norepinephrine. The group of $beta_1$ responses includes the positive inotropic effect on the heart, the lipolytic response of adipose tissue, and the inhibition of intestinal motility. Typical $beta_2$ responses include bronchodilation, glycogenolysis in skeletal muscle, and relaxation of the uterus. Although certain antagonists, such as practolol, have a somewhat greater potency at $beta_1$ receptors than at $beta_2$ receptors, there are no absolutely specific $beta_1$ or $beta_2$ antagonists known.

DOPAMINERGIC RECEPTORS

In addition to alpha- and beta-adrenergic receptors, the adrenergic nervous system contains dopaminergic receptors. Since these receptors are of known physiological importance only in the brain and perhaps in the renal vasculature, they are not considered in the discussion that follows.

PHARMACOLOGICAL CLASSIFICATION OF ADRENERGIC COMPOUNDS

During the last three decades hundreds of adrenergic agents have been synthesized. Functionally, these compounds can be classified as agonists, antagonists, or partial agonists. By definition, agonists are compounds that interact with adrenergic receptors and are capable of eliciting a maximal biological response (e.g., smooth muscle contraction). Adrenergic antagonists are compounds that interact with either alpha- or beta-adrenergic receptors and block agonist-induced biological responses either (a) by chemically altering the receptor or (b) by simple occupancy of the receptor thereby preventing access to the receptor by agonists. Partial agonists are compounds that interact with the receptor eliciting a biological response that is submaximal regardless of the concentration of partial agonist employed. Since, at high concentrations, partial agonists fully occupy the receptors but give only a partial response, they can have a partial antagonistic effect toward the stimulation by full agonists.

Every adrenergic agent can be functionally characterized by two properties —potency and intrinsic activity. The potency of a compound is related to the concentration of the compound required to elicit (in the case of agonists)

or antagonize (in the case of antagonists) a response. Thus the potency of a compound is determined by the affinity of the compound for the receptor sites. The intrinsic activity of a compound refers to the maximally attainable biological response that can be elicited by that compound. Thus, a full agonist has full intrinsic activity (arbitrarily set at unity), whereas an antagonist has zero intrinsic activity. Partial agonists have intermediate levels of intrinsic activity.

The discussion to follow subdivides adrenergic agonists and antagonists into alpha-adrenergic compounds and beta-adrenergic compounds. This subdivision was chosen in order to facilitate subsequent discussions of the structure-activity relationships of alpha- and beta-adrenergic receptors (Chapters 6 and 7). Some of the agonist compounds have activity as both alpha- and beta-agonists, but their orders of potency and structure activity relationships are different for the two responses. Most of the antagonist compounds are relatively specific for either alpha- or beta-adrenergic receptors. The following discussion is not intended to exhaustively consider all adrenergic agents but simply to focus on the main groups.

Alpha-Adrenergic Agonists

Most alpha-adrenergic agonists, including the endogenous catecholamines, are derivatives of beta-phenylethylamine (Fig. 2–4). A list of alpha-agonists commonly used in experimental work is provided in Table 2. The structures of many of these compounds are given in Table 2 and Chapter 6 or can be found elsewhere (4). The principal groups of these compounds are discussed below.

Catecholamines

The term "catecholamine" refers to phenylethylamine compounds that have hydroxyl groups at the 3 and 4 positions of the aromatic ring thus forming the "catechol" moiety. The presence of this moiety is required for maximal potency of the phenylethylamine as an alpha-agonist. The most potent catecholamine agonists are those that contain a hydroxyl group at the

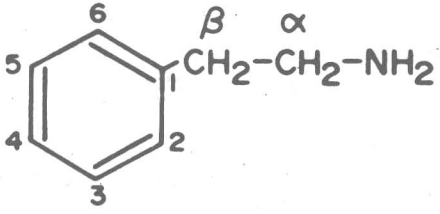

FIG. 2–4. The structure of phenylethylamine. Most adrenergic agonists are derivatives of this basic structure.

TABLE 2. *Some representative alpha-adrenergic compounds*

Alpha-adrenergic agonists	Alpha-adrenergic antagonists
Catecholamines	Imidazolines
Epinephrine	Phentolamine
Norepinephrine	Tolazoline
Isoproterenol	Haloalkylamines
Dopamine	Phenoxybenzamine
Nordefrin	Dibenamine
Other phenylethylamines	Ergot alkaloids
Phenylephrine	Ergotamine
Metaraminol	Ergocryptine
Hydroxyamphetamine	Ergocrystine
Methoxamine	Ergocornine
Ephedrine	Dihydroergotamine
Mephentermine	Dihydroergocryptine
Ergot alkaloids	Dihydroergocrystine
Ergonovine	Dihydroergocornine
Methysergide	Others
Ergotamine	Yohimbine
Others	Dibozane
Clonidine	Phenothiazines, e.g., chlorpromazine
	Butyrophenones, e.g., haloperidol

beta-carbon (see Fig. 2–4). The presence of this hydroxyl group creates a center of asymmetry around the beta-carbon, thus allowing two possible spatial conformations around this carbon. Numerous studies have shown that the stereoisomers of epinephrine and norepinephrine with the levo (−) configuration are about an order of magnitude more potent than the corresponding dextro (+)stereoisomers of these compounds. This striking stereospecificity suggests that a very specific three-dimensional conformation of the catecholamine is required for the maximal interaction with the receptor sites to occur. Dopamine, an analog of norepinephrine that lacks the asymmetry of the beta-carbon (because it has no beta-hydroxyl group), has a considerably lower potency as an alpha agonist. Isoproterenol (Fig. 2–3), a very potent beta-agonist, is a very weak alpha-agonist.

Noncatecholamine Phenylethylamines

Many of these compounds have been synthesized with various substitutions on the phenyl moiety. Some of the most commonly used compounds in experimental work are phenylephrine and metaraminol, which lack hydroxyl groups on the 4 position of the phenyl group. Both of these compounds are considerably less potent *in vitro* than epinephrine in eliciting alpha-adrenergic responses. Although these compounds are often considered relatively specific alpha-agonists, it should be noted that at high concentrations both phenylephrine and metaraminol interact with the beta-adrenergic receptor (5). A number of additional phenylethylamine compounds are listed