

# Adrenergic Receptors in the Autonomic Nervous System

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The early history of adrenergic receptors, encompassing the first half of this century, is closely associated with the adrenal gland and the investigation of the effects of its major catecholamine, adrenaline. Although the high concentration of adrenaline facilitated its chemical identification, this focus on the chromaffin tissue of the adrenal gland diverted attention from the rest of the sympathetic nervous system. It was not until 1948 that it was shown that sympathetic nerves released noradrenaline rather than adrenaline. Since then, the study of how these catecholamines interact with the effector cells of the sympathetic nervous system has led the way for other neural and humoral chemical transmitters. The aim of this chapter is to describe briefly some of the early history of adrenergic receptors and review the current state of knowledge about the major receptor subtypes (i.e.,  $\alpha_1$ ,  $\alpha_2$ ,  $\beta_1$ ,  $\beta_2$ ).

## TWO SYMPATHETIC TRANSMITTERS OR TWO RECEPTOR TYPES ?

By the beginning of this century, the work of Langley, Elliot, and others had identified adrenaline as the agent in extracts of adrenal medulla that contracted some smooth muscles, relaxed others, and raised blood pressure. In 1911, Cannon and de la Paz observed the similarity between the physiological reactions to stress mediated by the sympathetic nervous system (cardioacceleration, inhibition of gut motility) and the effects of adrenaline. In an attempt to reconcile the inhibitory and excitatory actions produced by the sympathetic nervous system, Cannon and Rosenblueth (1933) proposed that two chemical transmitters were released during sympathetic stimulation: sympathin I produced the inhibitory effects and sympathin E produced the excitatory effects. However, it was subsequently deduced that these two different responses were the result of

different adrenergic receptors, not different chemical transmitters.

Probably the earliest indication for multiple adrenergic receptors was the demonstration by Dale (1906) that ergot extracts blocked, and in some cases reversed, the effects of adrenaline or sympathetic nerve stimulation. Two studies published at the end of the 1940s provided the foundation for our current understanding of sympathetic transmission.

In 1948, Ahlquist presented evidence for two adrenergic receptors. He used six different catecholamines and determined their relative potencies in over 20 intact and isolated animal preparations from four different species. When the six test compounds were arranged in order of potency, the data segregated into two groups. In the first, designated as  $\alpha$ -receptor-mediated, adrenaline was the most potent, noradrenaline was the second most potent, and the synthetic agonist, isoproterenol, was the least potent (i.e., sixth in order). In the second group, designated as  $\beta$ -receptor-mediated, isoproterenol was most potent, adrenaline was second most potent, and noradrenaline was least potent.

This was probably the first time an agonist-based classification was used to demonstrate different receptor types. Ahlquist argued that adrenaline was the only sympathetic neurohormone and all the results of sympathetic stimulation could be explained in terms of adrenaline's action on either  $\alpha$ - or  $\beta$ -receptors. However, at the same time von Euler (1948) and others demonstrated that noradrenaline, not adrenaline, was the predominant catecholamine released from stimulated sympathetic nerves. The  $\alpha/\beta$  concept was further strengthened when Powell and Slater (1958) showed that dichloroisoproterenol was a selective antagonist/partial agonist of  $\beta$ -receptors. Ahlquist's work provided the model for future classifications of receptor subtypes from which derive current ideas about the four adrenergic receptors, their tissue distributions, and the responses they mediate (Table 2-1).

**Table 2-1** Tissue Distribution and Responses Mediated by Adrenergic Receptors

Receptor	Tissue	Response
$\alpha_1$	Smooth muscle: vascular, iris, radial ureter, pilomotor, uterus sphincters (gut, bladder)	Contraction
	Smooth muscle (gut)	Relaxation
	Heart	Positive inotropic ( $\beta_1 \gg \alpha_1$ )
	Salivary gland	Secretion
	Adipose tissue	Glycogenolysis
	Sweat glands	Secretion
	Kidney (proximal tubule)	Gluconeogenesis, $\text{Na}^+$ reabsorbed
$\alpha_2$	Presynaptic autoreceptor on sympathetic nerve endings	Inhibition of NE release
	Platelets	Aggregation, granule release
	Adipose tissue	Inhibition of lipolysis
	Endocrine pancreas	Inhibition of insulin release
	Smooth muscle (vascular)	Contraction
Kidney	Inhibition of renin release (?)	
$\beta_1$	Heart	Positive inotropic effect; positive chronotropic effect
	Adipose tissue	Lipolysis
	Kidney	Renin release
$\beta_2$	Liver	Glycogenolysis, gluconeogenesis
	Skeletal muscle	Glycogenolysis, lactate release
	Smooth muscle: bronchi, uterus, gut vascular (skeletal muscle) detrusor, spleen capsule	Relaxation
	Endocrine pancreas	Insulin secretion (?)
	Salivary gland	Amylase secretion

## $\alpha_1$ - AND $\alpha_2$ -RECEPTORS

Brown and Gillespie (1957) wanted to investigate the phenomenon of posttetanic facilitation (i.e., the increased response to a single stimulus that follows a high-frequency burst of stimuli) with a view to establishing its relationship to transmitter release. After treating a cat with dibenzylamine or dibenamine (irreversible  $\alpha$ -receptor antagonists), they found that the output of noradrenaline from the spleen at low stimulus frequencies increased about 5-fold. They explained these results by concluding that noradrenaline was metabolized on the adrenergic receptors, even though they also presented data that showed that these drugs had no effect on the metabolism of exogenous noradrenaline. With the availability of tritium-labeled noradrenaline, it has been possible to show that the release of noradrenaline is greater in the presence of  $\alpha$ -blockade and the increased overflow of transmitter is not due to reduced inactivation of transmitter (Kirpekar et al., 1973). Alpha blockade also inhibits the response of the cat spleen to sympathetic stimulation, so the question remained whether this inhibition was related to the increased noradrenaline release. This problem was resolved when it was shown that  $\alpha$ -blockade also increased stimulated noradrenaline release from cardiac tissue, where the sympathetic response is elicited primarily through  $\beta$ -receptors (Starke et al., 1971).

Langer (1974), considering that the concentrations of phenoxybenzamine needed to block the postsynaptic response differed from those required to enhance transmitter release, suggested that the postsynaptic receptors be referred to as  $\alpha_1$  and the presynaptic receptors be referred to as  $\alpha_2$ .

## ARE ALL $\alpha_2$ -RECEPTORS PRESYNAPTIC AUTORECEPTORS?

No! is the answer to this question. Exceptions to a subdivision of  $\alpha$ -receptors on the basis of location were soon discovered. Alpha-mediated responses with similar pharmacology to the presynaptic  $\alpha$ -receptor (i.e.,  $\alpha_2$ ) have been found on adipocytes, platelets, vascular smooth muscle, and pancreatic islets. These receptors are functional. For example, Drew and Whiting (1979) demonstrated vasoconstriction due to postjunctional  $\alpha_2$ -receptors.

Berthelsen and Pettinger (1977) suggested a classification on the basis of function:  $\alpha_1$  being excitatory and  $\alpha_2$  being inhibitory. However, classifying a receptor as inhibitory or excitatory is an arbitrary label dependent on the point of view. If binding of an agonist to an  $\alpha_2$ -receptor in blood vessels first inhibits an enzymatic activity, but this subsequently leads to vasoconstriction, then  $\alpha_2$ -receptors could be classified as either inhibitory or excitatory.

The current methods of subclassifying  $\alpha$ -receptors employ a combination of biochemical and pharmacological approaches. Selective agonists and antagonists have been developed that can be used to classify  $\alpha$ -adrenergic responses as either  $\alpha_1$ - or  $\alpha_2$ -receptor-mediated. A selection of these compounds is listed in Table 2-2. The biochemical approach to subclassification of  $\alpha$ -receptors is discussed in the section on signal transduction and second messengers.

## SIGNAL TRANSDUCTION AND SECOND MESSENGERS AS A MEANS TO CLASSIFY $\alpha$ -RECEPTORS

Even before the subclassification of  $\alpha$ -receptors, Hokin and Sherwin (1957) had shown that adrenaline stimulated the turnover of phosphatidylinositol (PI) in salivary gland. This effect was blocked by dibenamine and ergotamine, indicating that an  $\alpha$ -receptor was involved. Over the next 20 years, it was consistently found that increased phosphatidylinositol turnover stimulated by an adrenergic agonist was mediated by an  $\alpha$ -receptor (see Michell, 1975 for review). Tolbert et al. (1980) showed that prazosin was much more potent than yohimbine as an inhibitor of the adrenaline-induced increase in phosphatidylinositol turnover in isolated hepatocytes. This pointed to  $\alpha_1$ -receptor involvement in the response. Subsequent studies support the generalization that the primary response to activation

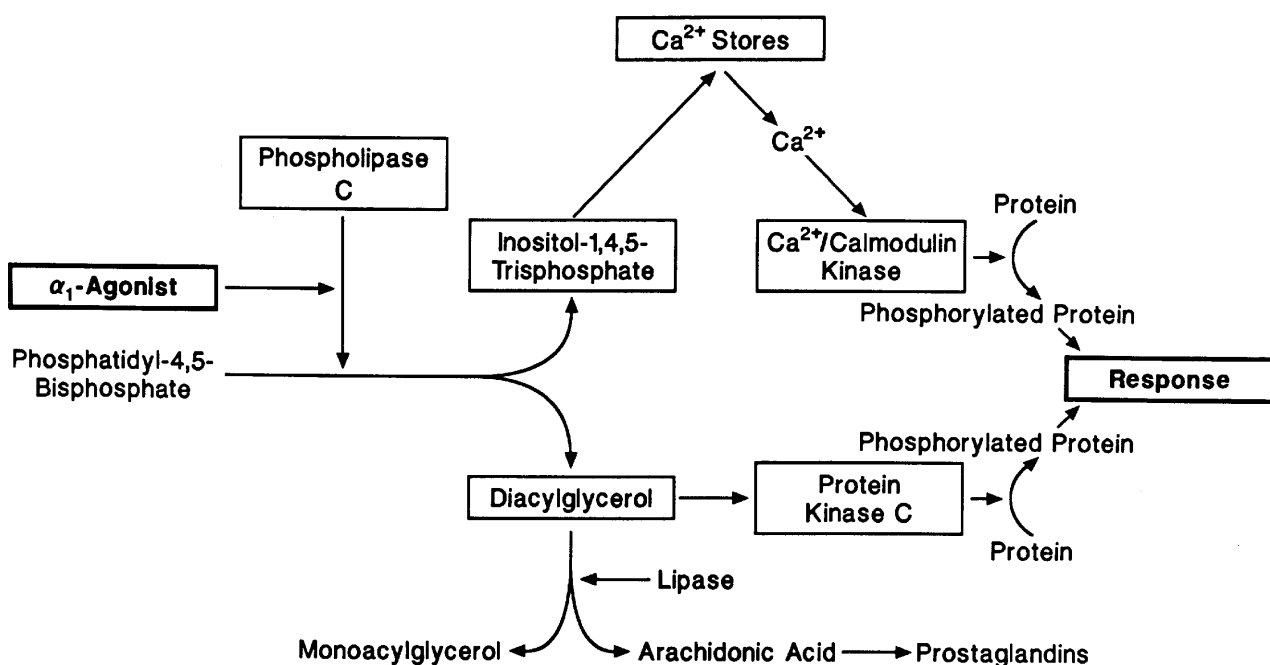
**Table 2-2** The Pharmacology of  $\alpha$ -Receptors

Receptor	Agonist	Antagonists
$\alpha_1$	Methoxamine Phenylephrine	Prazosin BE 2254 Corynanthine
$\alpha_2$	UK-14, 304 $\alpha$ -Methyl-NE Tramazoline Xylazine Clonidine <sup>a</sup>	Yohimbine Idazoxan Rauwolscine

<sup>a</sup>Partial agonist.

of  $\alpha_1$ -receptors is increased phosphatidylinositol turnover.

Michell (1975), in his review, noted that increased phosphatidylinositol turnover was often associated with calcium mobilization and deduced that the former preceded the latter. Our current understanding of how receptor-stimulated hydrolysis of phosphatidylinositol can lead to the generation of second messengers inside the cell is shown in Figure 2-1. It is unclear at the moment whether there is a G protein (i.e., a protein that binds guanine nucleotides; see Gilman, 1984 for review), analogous to those that link the other adrenergic receptors to adenylate cyclase, between the  $\alpha_1$ -receptor and the phosphodiesterase that cleaves phosphatidylinositol-4,5-phosphate to diacylglycerol and inositol-1,4,5-phosphate. The end result is liberation of two compounds that are potential second messengers.



**Figure 2-1** Pathways for hydrolysis of inositol phospholipids. (Adapted from Berridge, 1984, with permission of the author and publisher.)

The subsequent metabolism of inositol-1,4,5-phosphate becomes more complex as it is investigated, but the ultimate consequence is held to be release of calcium from internal stores such as the endoplasmic reticulum. Elevated intracellular calcium can then influence a range of cellular events, including vesicle-mediated secretion and contraction (in muscle cells), as well as increases in both synthesis and hydrolysis of cyclic nucleotides [cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP)]. The effects on cyclic nucleotide metabolism can be attributed in part to binding of calcium to the ubiquitous calcium-binding protein calmodulin.

Diacylglycerol on the other hand remains in the membrane and acts as an anchored binding site for protein kinase C (for reviews, see Nishizuka, 1983; Blackshear et al., 1988). Protein kinase C activation also requires calcium and phosphatidylserine as co-factors. The tumor-promoting phorbol esters also stimulate protein kinase C. Activation of protein ki-

nase C also seems to lead to its movement to a plasma membrane compartment and a parallel reduction in the concentration of soluble protein kinase C. Even while membrane bound, this kinase can still phosphorylate cytoplasmic proteins such as myosin light chains. As a further complication, diacylglycerol can be metabolized by one of two possible routes resulting in the liberation of arachidonic acid (which is usually esterified at the 2-position in diacylglycerol), from which prostaglandins can then be synthesized (see Berridge, 1984 for review).

The idea that  $\alpha$ -adrenergic activation can lead to the inhibition of adenylate cyclase activity and reduced synthesis of cAMP also predates the  $\alpha_1/\alpha_2$  subclassification (Robison et al., 1972). Adipocytes respond to adrenaline with an increase in cAMP synthesis (i.e., a  $\beta$ -receptor-mediated effect). The cAMP accumulated in response to a  $\beta$ -selective agonist (e.g., isoprenaline) or to adrenaline in the presence of an  $\alpha_2$ -antagonist is up to 10-fold higher. Although  $\alpha_2$  activation will lower basal levels of cAMP

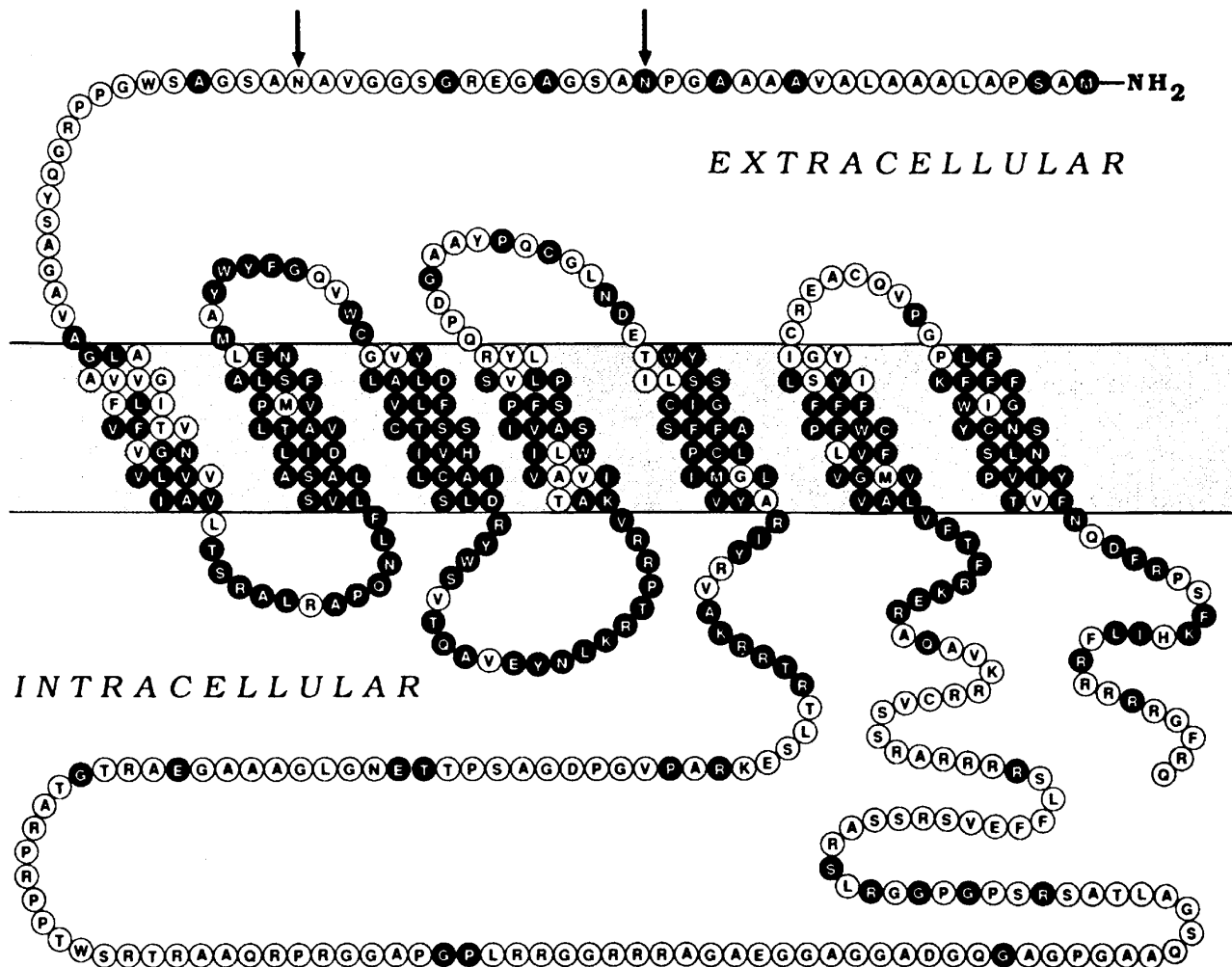


Figure 2-2 Deduced structure of the  $\alpha_2$ - and  $\beta_2$ -adrenergic receptors. (The illustration on the left of the  $\alpha_2$ -receptor sequence was taken from Regan et al., 1988, and the  $\beta_2$ -receptor sequence on the right was taken from Dohlmán et al., 1987, with permission of the authors and publishers.)

synthesis, this is only by a factor of two. Thus,  $\alpha_2$ -receptors are more effective at inhibiting stimulated than basal cAMP synthesis. In addition, this effect is not restricted to adrenergic mechanisms. Forskolin activates adenylate cyclase directly, apparently without a G protein intermediary, yet  $\alpha_2$  activation significantly inhibits its action (Burns et al., 1982).

$\alpha_2$ -Receptors appear to be similar to other receptors that are negatively coupled to adenylate cyclase in that they lower the maximum rate of cAMP synthesis but do not affect the affinity for substrate. The precise mechanism by which  $\alpha_2$ -receptors inhibit cyclase has not been elucidated, though it appears that guanosine triphosphate (GTP) is necessary for function.

### ARE THERE MORE THAN TWO $\alpha$ -RECEPTOR SUBTYPES?

The  $\alpha_1/\alpha_2$  nomenclature continues to be a valuable classification scheme, but arguments have been made for further subdivisions of  $\alpha$ -adrenergic receptors. These arguments have been based on agonist affinities, antagonist affinities, requirement for extracellular calcium rather than mobilization of intracellular calcium stores, and linkage to PI turnover (see Han et al., 1987 and Flavahan and Vanhoutte, 1986 for reviews). The subject is made confusing by the usage of subscript suffixes (e.g.,  $\alpha_{1H}$ ,  $\alpha_{1L}$ ;  $\alpha_{1a}$ ,  $\alpha_{1b}$ ;  $\alpha_{1A}$ ,  $\alpha_{1B}$ ) that are neither consistent nor equivalent. Further investigations are needed to determine

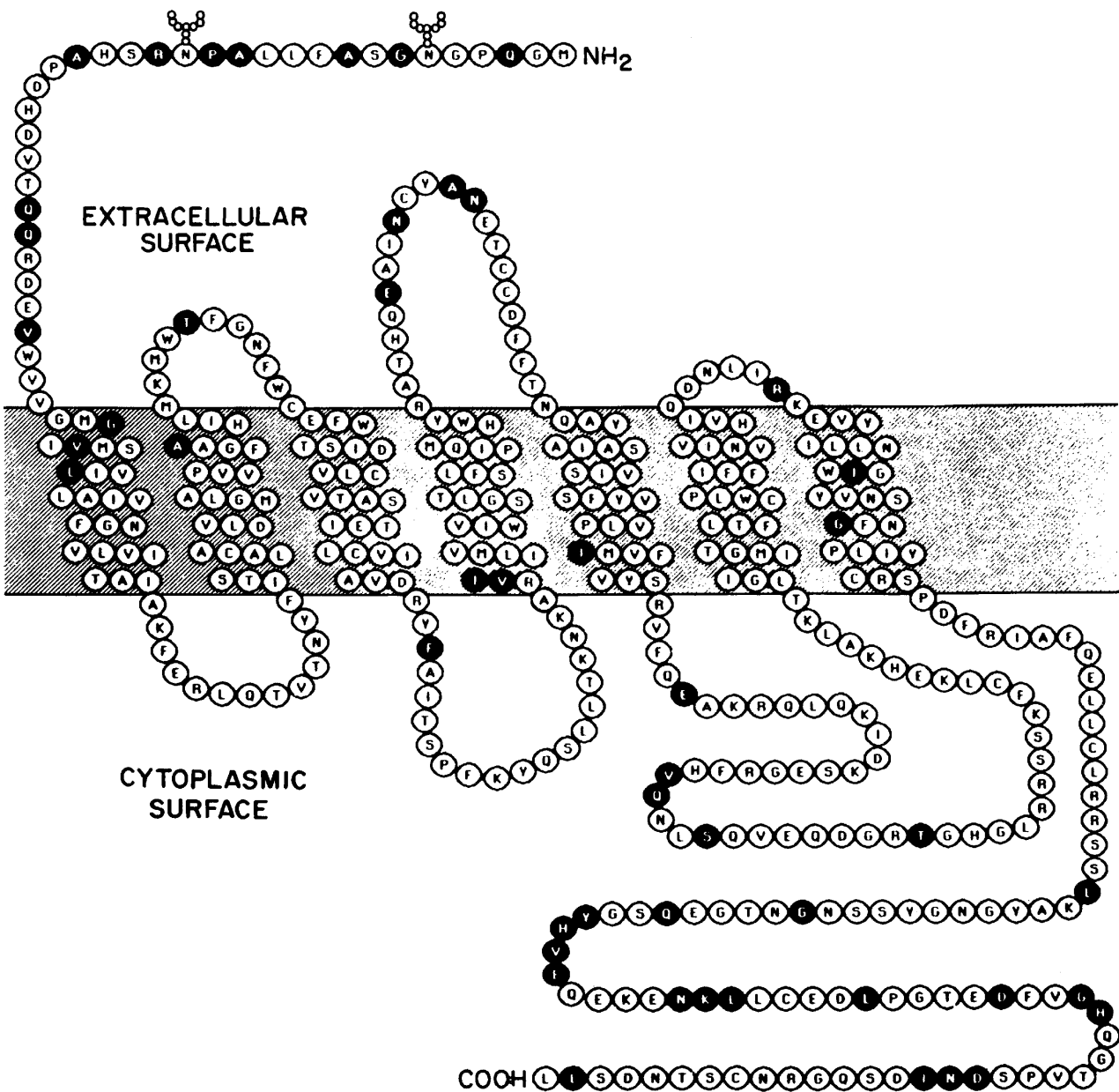


Figure 2-2 (Continued)

whether greater subclassification of  $\alpha_1$ -receptors is needed, but the reader should keep this possibility in mind.

High- and low-affinity states of  $\alpha_2$ -receptors have been described and are expected from the linkage of these receptors to G proteins (see below). There is, however, evidence for more than one kind of  $\alpha_2$ -receptor. Subtypes of  $\alpha_2$ -receptors have been suggested on the basis of differential affinity for the antagonist rauwolscine (Alabaster et al., 1986). Heterogeneity in  $\alpha_2$ -receptors is supported by the discovery of two genes (one expressed in platelets, the other in the kidney) that are localized to different chromosomes (Regan et al., 1988). Inhibition of cAMP synthesis does not appear to reduce norepinephrine release (see Bylund and U'Prichard, 1983 for review). Thus, there is the possibility that there is a third gene encoding the presynaptic  $\alpha_2$ -autoreceptor.

There is the potential for a number of  $\alpha$ -receptor subtypes. Some of these problems may be resolved when the receptor proteins from the different tissues are cloned and their amino acid sequences are compared (Figure 2-2).

### $\beta$ -RECEPTORS STIMULATE ADENYLATE CYCLASE AND ELEVATE INTRACELLULAR cAMP

Sutherland and co-workers (reviewed in Sutherland et al., 1965) uncovered the link between adrenergic stimulation, cAMP synthesis, and hepatic glycogenolysis. The cAMP produced inside the cell activates protein kinase A, which in turn phosphorylates phosphorylase kinase. This step activates phosphor-

ylase kinase, which now converts inactive glycogen phosphorylase to the active form, resulting in the breakdown of glycogen into glucose-1-phosphate (Figure 2-3). The increase in cAMP also inhibits the synthesis of glycogen by stimulating the phosphorylation of an inhibitor that then binds to and inhibits a phosphatase. The role of the phosphatase is to dephosphorylate both phosphorylase kinase (to inactivate it) and glycogen synthase (to activate it).

From this work, Sutherland and co-workers suggested that activation of  $\beta$ -receptors increases cAMP synthesis by stimulating adenylate cyclase. The major components of this system have been isolated (see Gilman, 1984 for review) and are shown in Figure 2-4. As a result of the binding of agonist, the receptor undergoes a conformational change that allows it to bind to a G protein complex, in this case  $G_s$ , because adenylate cyclase will ultimately be stimulated. The G protein is composed of three subunits:  $G_\alpha$ ,  $G_\beta$ , and  $G_\gamma$ .  $G_\alpha$  also has bound GDP in the absence of agonist. After the agonist-receptor complex binds to the G protein complex, the GDP is replaced by GTP and  $G_\alpha$  dissociates from the complex. This activated form of  $G_\alpha$  now binds to and stimulates the catalytic subunit of adenylate cyclase to synthesize cAMP from ATP. When the GTP is hydrolyzed to GDP,  $G_\alpha$  dissociates and now reforms the G protein complex with the other G subunits to await further binding to the agonist-receptor complex.

This process is not unique to  $\beta$ -receptors. In the liver, for example, glucagon stimulates cAMP synthesis through the same pool of  $G_s$  complex and adenylate cyclase, though it binds first to a different receptor protein. This is one reason why the response of a cell to adrenergic agonists can be affected by ex-

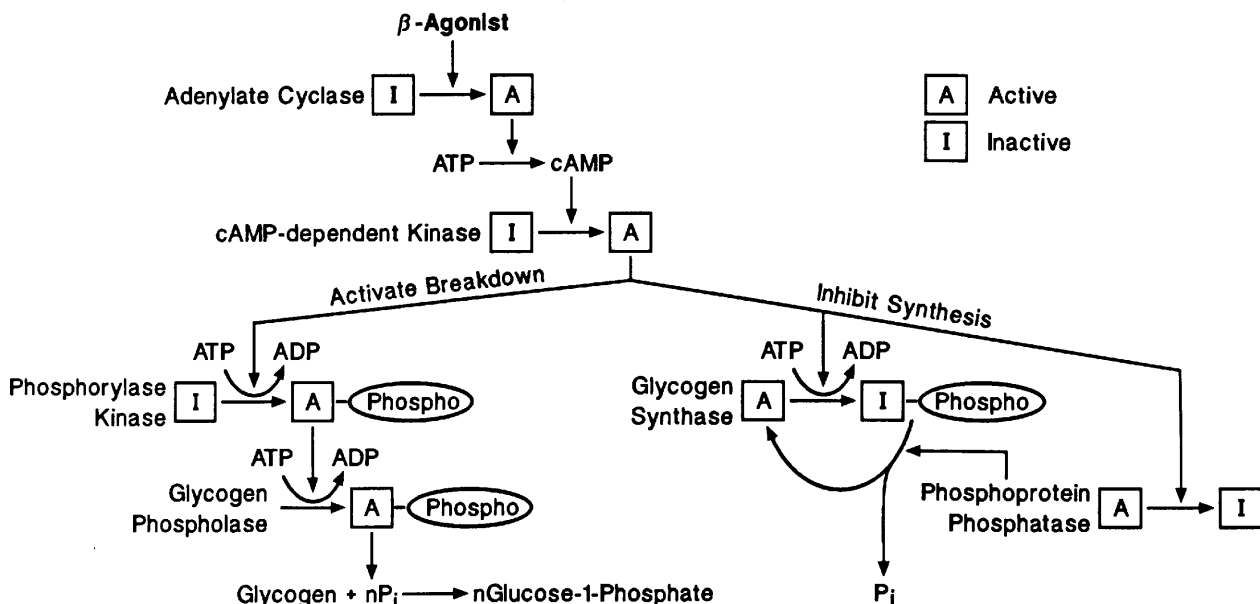


Figure 2-3 The regulation of glycogen metabolism by  $\beta$ -agonists through cAMP.

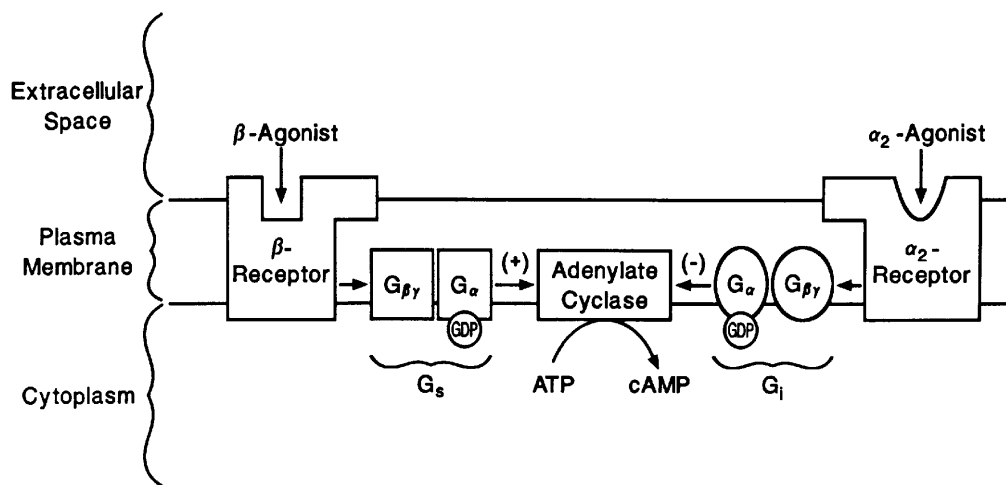


Figure 2-4 The components of the adenylate cyclase complex. (Adapted from Gilman, 1984.)

posure to other agents (see below). Before leaving the subject of adrenergic modulation of glycogenolysis, it is worth mentioning that the hyperglycemia produced by adrenergic agonists may not be simply due to activation of  $\beta$ -receptors in the liver. Adrenergic agonists can also increase the supplies of glucose by inhibiting the release of insulin, increasing the release of glucagon, and increasing the synthesis of glucose from lactate in skeletal muscles. There is also evidence that  $\alpha$ -mediated glycogenolysis can be elicited from the liver, without changing cAMP levels. It has been claimed that this is the predominant mechanism in some species (Exton, 1985).

### $\beta_1$ - AND $\beta_2$ -RECEPTORS

In a short report to *Nature (London)* in 1967, Lands et al. demonstrated that the actions of noradrenaline that had been assigned to  $\beta$ -receptors in Ahlquist's classification could be further subdivided. This work was also an agonist-based division, comparing the potencies of ten catecholamine derivatives in four tissue preparations. They showed that noradrenaline and adrenaline had similar affinities at  $\beta_1$ -receptors. The affinity of noradrenaline for  $\beta_2$ -receptors was similar to its affinity for  $\beta_1$ -receptors whereas the affinity of adrenaline was nearly 100-fold higher at  $\beta_2$ -receptors. This study laid the foundation for the development of a large array of selective agonists and antagonists for research and clinical use. A selection of these compounds is listed in Table 2-3.

The distribution of the  $\beta$ -receptor subtypes varies. Although one type may predominate in an organ or tissue, it is not unusual to find both types. The heart contains predominantly the  $\beta_1$  types, which are responsible for both inotropic and chronotropic responses, but there are also  $\beta_2$ -receptors that may be linked to the inotropic response.

The  $\beta_1$ - and  $\beta_2$ -receptors are very similar; both stimulate cAMP synthesis and both are glycosylated proteins that have identical mobilities when analyzed by electrophoresis in polyacrylamide gels. Peptide maps of the two receptors are also very similar (see Dohlman et al., 1987, for review). These observations raise the question of whether there are two different proteins, a common precursor that is modified in some way to yield one or other activity, or a single protein that is regulated in some way by its environment.

Molecular biological investigations have provided the answer to this question. Complementary DNA clones coding for  $\beta$ -receptors from several species have been isolated (Figure 2-2). These show that the  $\beta_1$ - and  $\beta_2$ -receptors are homologous, though different, proteins. The  $\beta$ -receptor cDNAs also show high homology with other G protein-coupled receptors, including rhodopsin (a receptor for "light"), the  $\alpha_2$ -receptor, and some types of muscarinic cholinergic receptors (Dohlman et al., 1987). Common features of these proteins include seven  $\alpha$ -helical membrane spanning regions connected by intra- and extracellular loops. There is a high degree of homology be-

Table 2-3 The Pharmacology of  $\beta$ -Receptors

Receptor	Agonist	Antagonists
$\beta_1$	Prenaterol	ICI 89,407 CGP 26,505 Betaxolol Atenolol Practolol Metoprolol
$\beta_2$	Terbutaline Salbutamol Rimiterol Adrenaline	ICI 118,551 IPS 339

tween these transmembrane regions in  $\beta_1$ - and  $\beta_2$ -receptors. Thus, these regions are probably involved in some common function such as binding to G proteins. Now that the  $\beta$ -receptor proteins have been cloned and expressed in cell lines that do not normally express them, we can expect that selective mutation experiments will reveal the agonist-binding sites and show why  $\beta_1$ - and  $\beta_2$ -receptors differ in their affinity for selective agonists and antagonists.

## REGULATION OF ADRENERGIC RECEPTOR FUNCTION

Is there regulation of signaling in the sympathetic nervous system? In the face of fixed numbers of adrenergic receptors on effector cells, the concentration of catecholamine, whether acting as a neurotransmitter at a synapse or as a hormone, will determine the magnitude of the response. But there is good evidence that the adrenergic receptors are also dynamically regulated in response to the actions of adrenergic agonists and also to other agents. The regulation of signal transduction through  $\beta$ -adrenergic receptors has been most extensively studied, so they will be discussed first.

When a tissue containing  $\beta$ -receptors is first exposed to a  $\beta$ -agonist, the synthesis of cAMP increases due to activation of adenylate cyclase. After about 30 minutes the rate of cAMP synthesis declines and the tissue is said to be desensitized. If the response to only  $\beta$ -agonists is attenuated, then this is referred to as *agonist-specific* or *homologous desensitization* (see Sibley et al., 1987, for review). The activation of adenylate cyclase by agonists at other receptors or by nonreceptor activators such as fluoride ion or forskolin is not diminished. The mechanism of desensitization appears to involve both a reduction in the number of receptors on the cell surface and an uncoupling of the receptors from adenylate cyclase.

The mechanism underlying homologous desensitization appears to involve phosphorylation of the receptor. Phosphorylation begins within minutes of agonist occupation and leads to incorporation of about 2 mol of phosphate per mol of receptor. The phosphorylation does not appear to involve cAMP or require the coupling of receptor to  $G_s$  (Strasser et al., 1986). Rather, there is evidence for a cytosolic kinase ( $\beta$ -adrenergic receptor kinase) that appears to phosphorylate only the agonist-occupied receptor. Presumably the conformational change subsequent to agonist occupation reveals a phosphorylation site that would be otherwise hidden from the kinase. Phosphorylation of  $\beta$ -receptors uncouples them from the  $G_s$  protein (Sibley et al., 1986), impairing their ability to stimulate cAMP synthesis. Phosphor-

ylation also promotes internalization of  $\beta$ -receptors into intracellular compartments that are not yet fully characterized. In this compartment, a phosphatase appears to dephosphorylate the receptor so it can return to the cell surface (Sibley et al., 1986).

Desensitization of  $\beta$ -receptors can also occur in the absence of agonist. This is termed *heterologous desensitization* and also involves phosphorylation of the receptor but not internalization (see Sibley et al., 1987 for review). In part, the diminished response to agonists involves uncoupling of receptor from adenylate cyclase, and this can be correlated with cAMP-dependent receptor phosphorylation (Sibley et al., 1984b). Since the response to stimulation of other receptors and to agents that bypass receptors is also diminished, then there must be changes in the adenylate cyclase complex as well. Function impairment of  $G_s$  and increased activity of  $G_i$  have both been observed in association with heterologous desensitization of  $\beta$ -receptors (Kassis and Fishman, 1982; Garrity et al., 1983). Phosphorylation of  $\beta$ -receptors by cAMP-dependent kinases and protein kinase C appears to take place on the same serine residues (Bouvier et al., 1986). Muscarinic stimulation in the heart can lead to  $\beta$ -receptor desensitization (Limas and Limas, 1985). Some of the muscarinic receptors in heart are linked to elevated phosphatidylinositol breakdown, and this, in turn, leads to stimulation of protein kinase C. Therefore, this sequence of events illustrates how activity at one receptor (the muscarinic receptor) can modify the responsiveness of another receptor (the  $\beta$  receptor).

Although the discussion of receptor regulation has focused on  $\beta$ -receptors, there is also evidence that  $\alpha$ -receptors are subject to regulation. It is known that  $\alpha_2$ -receptors, and probably  $\alpha_1$ -receptors also, are coupled to the next stage of signal transduction by G proteins, so the description of heterologous desensitization already given can be applied. It has been shown that  $\alpha_1$ -agonists promote  $\alpha_1$ -receptor desensitization that can be correlated with receptor phosphorylation and sequestration, i.e., homologous desensitization (Leeb-Lundberg et al., 1987). Although progress on the molecular biology of  $\beta$ -receptors has been ahead of that on the other adrenergic receptors, the gap is constantly being narrowed. We can soon expect to know as much about regulation of signaling through  $\alpha$ -receptors as is known about that through  $\beta$ -receptors.

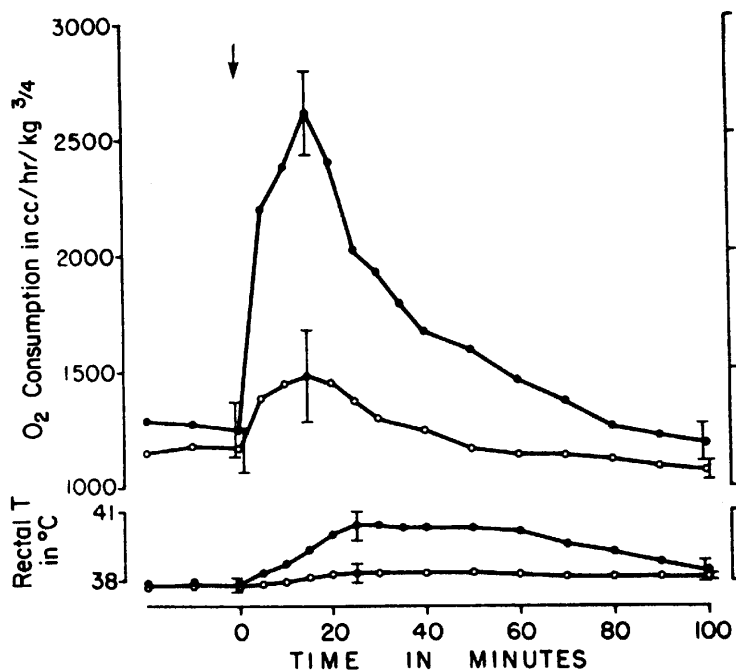
So far in this section, short-term regulation of adrenergic receptor function has been discussed. The regulation of adrenergic responsiveness over periods of days and weeks will now be considered. The relationship between circulating levels of thyroid hormone and the expression of  $\beta$ -receptors is a good example that has both physiological and clinical relevance.

Patients with pathological elevations of circulating thyroid hormones show manifestations typical of adrenergic activation: tachycardia, increased thermogenesis, and sweating (Bilezikian and Loeb, 1983). These symptoms can be successfully treated with  $\beta$ -receptor antagonists. Experimental studies point to increased numbers of  $\beta$ -receptors in some, though not all, tissues during thyrotoxicosis. Interestingly,  $\alpha$ -receptor numbers appear to be reduced. Hypothyroid patients show some symptoms that could be attributed to impaired adrenergic function. In hypothyroidism, there appear to be fewer  $\beta$ -receptors.

Changes in the number of receptors on the cell surface cannot be extrapolated directly to an increased response of that cell to agonist because there may be "spare" receptors. The term *spare receptors* embodies the concept that the maximum biological response of a cell can be elicited when less than 100% of the receptors are occupied by agonist. Let us assume that there are 1,000 receptors per cell and the maximum increase in cAMP synthesis is elicited by only 10% occupancy: i.e., the maximum response is obtained when 100 receptors have bound agonist. What will be the consequence of increasing the number of receptors by 50%? The result is a shift in the dose-response curve to the left, i.e., the same effect as increasing the affinity for agonist. Direct assessment of agonist binding, for example, radioligand binding studies, would show increased numbers of binding sites but no change in affinity. The increased responsiveness at low concentrations can be demonstrated only by measuring the functional consequences of receptor occupancy. Conversely, if receptor concentration were reduced, the result would depend on the magnitude of the re-

duction. If the number of receptors did not fall below our hypothetical value of 100 receptors per cell needed to produce the maximum response, then we would see a right shift of the dose-response curve. Larger reduction would, however, lead to a reduction also in the maximum response that could be elicited. Thyroid hormone can regulate the level of expression of adrenergic receptors. This regulation is of physiological relevance during cold acclimation. Exposure of humans to a cold environment for several days results in increased serum levels of thyroid hormone. A part of the hypothalamus appears to be sensitive to core body temperature. If temperature falls, presumably the release of thyrotropin releasing hormone from the neurons in the hypothalamus and into the hypophyseal portal system is increased. This stimulates the release of thyrotropin from anterior pituitary cells, which in turn stimulates the synthesis and secretion of thyroid hormones from the thyroid gland. As already discussed, the response to adrenergic stimulation is then increased, in part by increasing the numbers of  $\beta$ -receptors. The effect of cold acclimation on the response to sympathetic stimulation is shown in Figure 2-5, taken from the work of Hseih and Carlson (1957). Administration of noradrenaline to a warm acclimated rat produces only a small increase in oxygen consumption and rectal temperature. After exposure to 5°C for 3-4 weeks, the same dose of noradrenaline produces much larger effects on oxygen consumption and rectal temperature.

Other hormones can also change the number of adrenergic receptors. In experimental studies  $\alpha_1$ -adrenergic receptors on the myometrial cells of the rabbit uterus increased with elevation of plasma con-



**Figure 2-5** Effect of cold acclimation on noradrenaline-stimulated thermogenesis. (○, warm acclimated; ●, cold acclimated). Noradrenaline (0.2 mg/kg) was administered at the time indicated by the arrow. (From Hseih and Carlson, 1957, with permission of the authors and publisher.)

centrations of estrogens. This was accompanied by increased response to agonist. At the same time the number of  $\alpha_2$ -receptors on platelets fell, providing a possible explanation for the increased incidence of thromboembolic disease in individuals receiving estrogens (e.g., women taking oral contraceptives).

In summary, the magnitude of the response of a tissue during sympathetic stimulation depends on several factors: previous exposure to adrenergic agonists (homologous desensitization), the biochemical state (heterologous desensitization), and the endocrine state of the individual.

## SUMMARY

Considerable advances have been made in the last 100 years in our understanding of how the sympathetic nervous system elicits a response from its target tissues. Four concepts stand out that are important to understanding the physiological function of the sympathetic nervous system: (1) there are at least four receptor types,  $\alpha_1$ ,  $\alpha_2$ ,  $\beta_1$  and  $\beta_2$ , (2) these receptors allow norepinephrine and adrenaline to elicit different responses by virtue of their linkage to different biochemical processes, (3) adrenaline is more potent than noradrenaline at all of these receptors, though it is most potent at  $\beta_2$ -receptors, and (4) most tissues contain more than one adrenergic receptor, so the response will depend on the agonist.

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