THE β-ADRENERGIC RECEPTOR AND ITS MODE OF COUPLING TO ADENYLATE CYCLASE

Author: **Alexander Levitzki**
Department of Biological Chemistry
The Institute of Life Sciences
The Hebrew University of Jerusalem
Jerusalem, Israel

Referee: Ernst J. M. Helmreich
Department of Physiological Chemistry
University of Würzburg
Würzburg, Federal Republic of Germany

I. INTRODUCTION

Catecholamines are synthesized and stored in nerve terminals in both the peripheral and the central nervous system. Upon release of the catecholamines, they interact with specific postsynaptic receptors, triggering a wide variety of cellular processes which depend on the target cell. l-Norepinephrine (l-noradrenaline) and l-epinephrine (l-adrenaline) are also synthesized in the adrenal medulla and, when released from the gland into the bloodstream, reach a variety of tissues on which they again act as hormones. The interaction of the catecholamine with the receptor triggers a primary biochemical reaction executed by a catalytic apparatus coupled to the catecholamine receptor. The type of primary catalytic event triggered by the binding of ligand to the receptor depends either on the activation of adenylate cyclase or on the opening of a gate for Ca^{++} ions, based upon the type of receptors. Other events that follow the binding of an agonist to the receptor have been encountered as well; for example, the change in membrane potential and the activation of phosphatidylethanolamine methylation as a result of β-receptor occupancy. The roles of the two latter events have not yet been fully delineated.

Subsequent to the triggering reactions, a cascade of biochemical reactions is further triggered, eventually yielding the final physiological response of the cell and the tissue. In this review we shall exclusively analyze the current knowledge of the β-adrenergic receptor and its mode of coupling to the enzyme adenylate cyclase. However, before we dive into the known and unknown facts of β-receptors, we shall outline very briefly the types of catecholamine receptors known and summarize the types of biochemical events identified as belonging to these receptors. In this review we do not intend to discuss the fine details of ligand specificity of the different subclasses of β-adrenergic receptors, since many excellent reviews on this subject have been recently published in the pharmacological literature. An attempt will not be made to cite every paper published in this field for two reasons. First, retrieval computer systems are now available to almost every scientist, and no review can be as up to date as those. Secondly, and most importantly, by the time this review is published, new information will already accumulate and the reference list will be outdated. Therefore, this paper will cite only the most relevant publications that appeared by August 1979. The review will be further restricted to the biochemical aspects of the β-receptor-cyclase interactions.

II. CLASSIFICATION OF CATECHOLAMINE RECEPTORS

Dale, in the beginning of this century, already recognized the existence of multiple types of adrenergic receptors. He saw that certain effects of epinephrine can be blocked by ergot alkaloids, whereas others remain unaffected. For example, ergot alkaloids inhibit the l-epinephrine-induced contraction of certain muscle organs. On the other hand, the same epinephrine preparation continued to exert another type of effect — namely, relaxation — even in the presence of ergot. In fact, the latter effects became more prominent once ergot was present. The antagonistic effects of epinephrine (Table 1) on the same target tissue suggested the existence of two types of receptors. These observations were extended and analyzed in detail by Ahlquist. Ahlquist, in the 1940s, demonstrated, using a large selection of phenylethylamine derivatives, that the two types of responses (Table 1) respond differently to the same family of compounds — namely, the potency ratios of the phenylethylamines in the two types of responses are diametrically opposed. This finding led to the definition of α- and β-adrenergic receptors[1] as follows.

α-Adrenergic receptors are those receptors that mediate the response to catecholamines with the potency order: (−) epinephrine > (−)norepinephrine > (-) α-methylnorepinephrine > (−)α-methylepinephrine > (−)isoproterenol (Figure 1). The stimulatory effect of these ligands is blocked competitively by phentolamine, dibenamine, and phenoxybenzamine (Figure 2).

β-Adrenergic receptors respond with agonist specificity of: (−)isoproterenol > (−)epinephrine > (−)α-methylepinephrine > (−)α-methylnorepinephrine > (−)norepinephrine (Figure 1). The β-stimulatory effect of catecholamines is blocked competitively by (−)propranolol, (−)alprenol, (−)pindolol, and (−)hydroxybenzylpindolol (Figure 2).

The classification into α- and β-receptors has been extended in recent years, as a result of very extensive studies on the structure-function relationship. For instance, β-adrenergic responses can be divided into two subclasses: β_1 and β_2. Epinephrine and norepinephrine are approximately equipotent agonists for β_1-receptors which are preferentially blocked by practolol. Epinephrine is more potent than norepinephrine in activating β_2-receptors which are inhibited by butoxamine. A variety of antagonists such as propranolol, alprenolol, and pindolol inhibit competitively, in an identical fashion, the catecholamine response of both β_1- and β_2-receptors. Similarly, α-adrenergic receptors can be divided into two subclasses: α_1 and α_2. Again, the ligand specificity of the two subtypes of α-receptors is different. α_1-Receptors are the characteristic postsynaptic $\alpha-$ adrenergic receptors, whereas α_2- receptors are the presynaptic autoreceptors in catecholaminergic synapses. A good summary of some aspects of the subclassification of adrenergic receptors was published recently.[2] Both α- and β-adrenergic receptors are stereospecific for the R stereoisomer for both the agonist and the antagonist.

A third major class of catecholamine receptors are the dopamine receptors which occur both in the central nervous system and in the periphery. These receptors occur at dopaminergic nerve terminals, and the agonist specificity is dopamine≫ norepinephrine. The dopaminergic response is blocked competitively by specific antagonists such as phenothiazines, butyrophenones (Figure 2), and butaclamol. It is presently quite clear that there are two classes of dopamine receptors: D1 receptors and D2 receptors which differ in their ligand specificities. Also, the D1 receptor is coupled to adenylate cyclase, whereas the D2 receptor is not. A summary on the current status of dopamine receptors has recently been published.[3] It should be emphasized, however, that lysergic acid derivatives interact with dopamine receptors as well as with α-receptors. Thus, ergocryptine was found to be an α-blocker, while d-LSD and bromocryptine were

Table 1
TYPICAL PHYSIOLOGICAL ACTIONS OF α- AND β-ADRENERGIC RECEPTORS

System or tissue	Action	Receptor
Cardiovascular system		
heart	Increased force of contraction	β
	Increased rate	α
Blood vessels	Constriction	α
	Dilation	β
Respiratory system, tracheal, and bronchial smooth muscle	Relaxation	β
Iris (radial muscle)	Pupil dilated	α
Smooth muscle, uterus	Contraction	α
Spleen	Relaxation	β
	Contraction	α
Bladder	Contraction	α
	Relaxation	β
Skeletal muscle	Changes in twitch tension	β
	Increased release of acetylcholine	α
	Increased glycogenolysis	β
Adipose tissue	Increased lipolysis	β

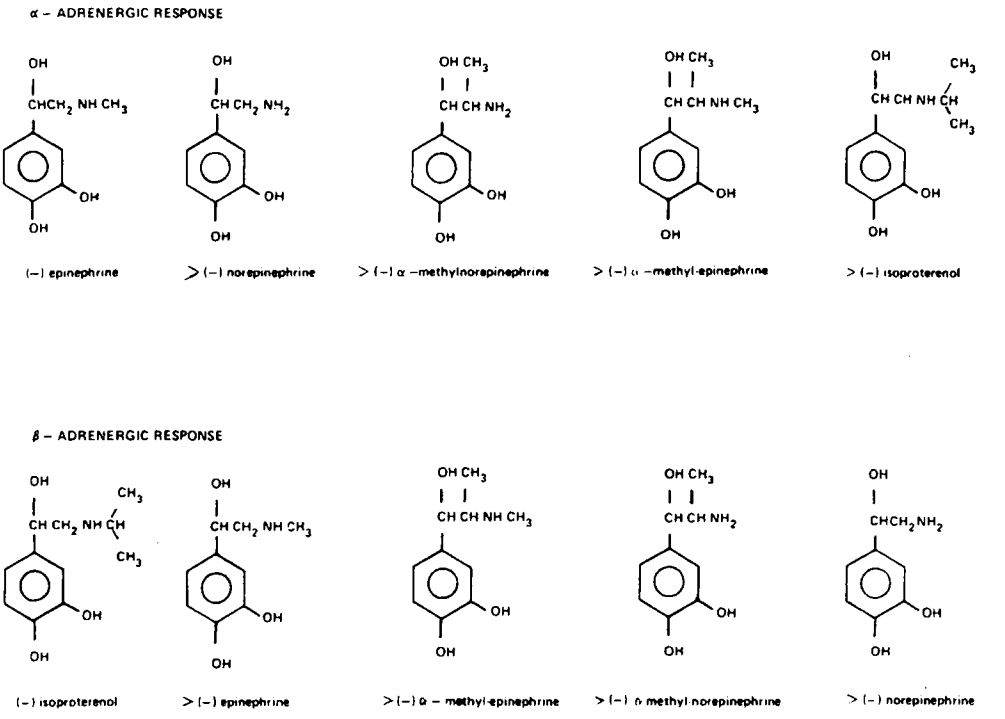


FIGURE 1. α- and β-adrenergic agonists.

found to bind to dopamine receptors. Also, certain antidopaminergic drugs such as butyrophenones and phenothiazines bind to α-adrenergic receptors and therefore cause unwarranted clinical side effects in patients who have to be treated with antidopaminergic drugs.

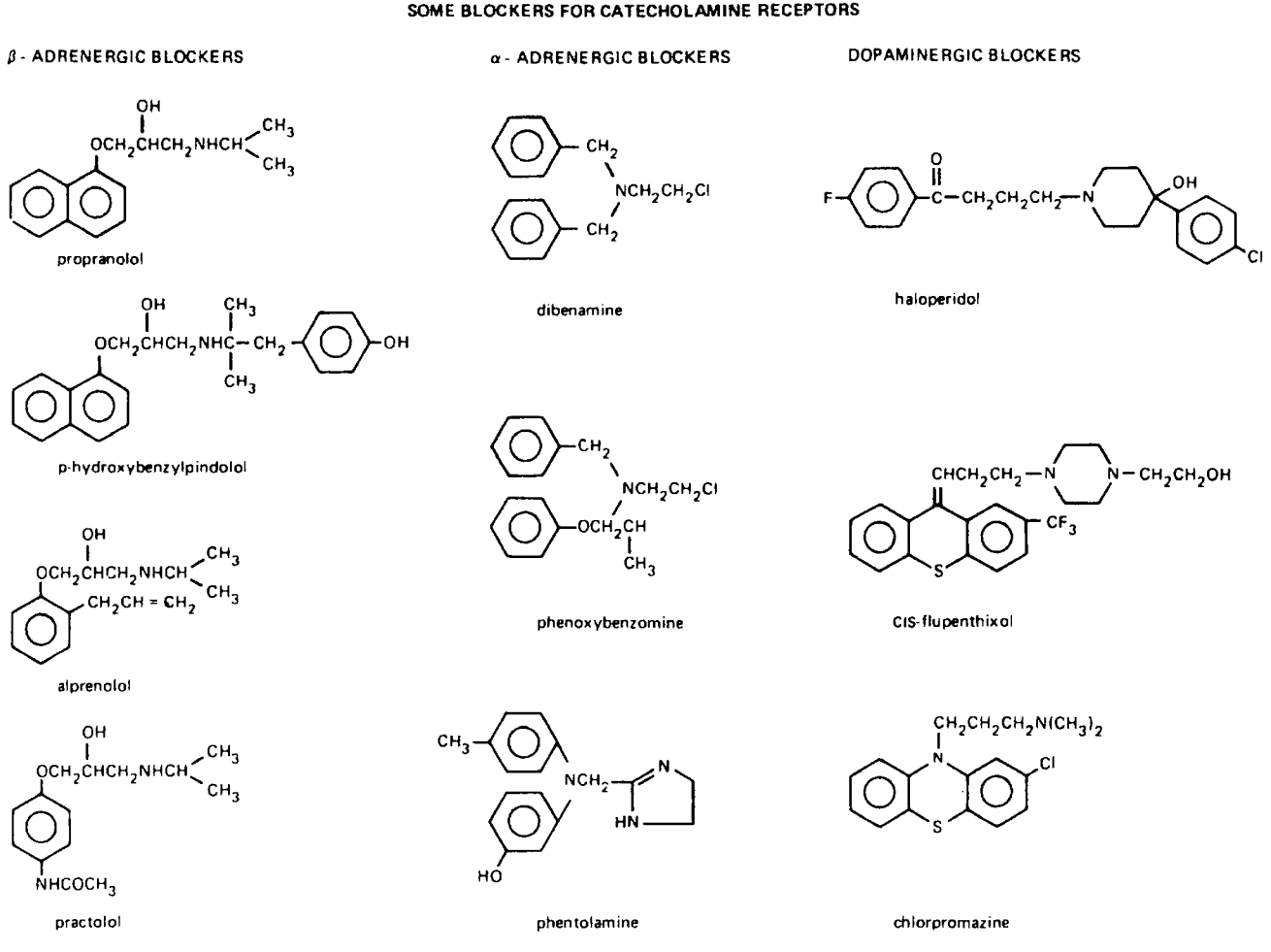


FIGURE 2. Typical α-adrenergic, β-adrenergic, and dopaminergic blockers.

III. BIOCHEMICAL SIGNALS COUPLED TO CATECHOLAMINE RECEPTORS

A. β-Adrenergic Receptors

Of all catecholamine receptors, the β-adrenergic receptors have been given the most attention. This may be due to the fact that the primary biochemical signal elicited upon agonist binding to the surface β-receptors has been identified and found to be the activation of adenylate cyclase producing the "second messenger" cAMP from ATP within the target cell.[4]

$$\text{ATP} \xrightarrow{\text{1-catecholamine}} \text{cAMP} + \text{PPi} \qquad (1)$$

In this respect, the coupling between the β-adrenergic receptor and the enzyme adenylate cyclase is similar to the coupling between adenylate cyclase and hormone receptors to certain polypeptide hormones such as glucagon, ACTH, and secretin. In certain cells such as the liver cell and the fat cell, β-adrenergic receptors as well as receptors for polypeptide hormones are coupled to the enzyme adenylate cyclase. The second messenger cAMP produced intracellularly by the enzyme adenylate cyclase triggers a large variety of biochemical events typical to the cell, usually through the activation of protein kinase, as a first step. The activation of adenylate cyclase by β-adrenergic agonists is mediated by the nucleotide GTP which acts in a synergistic fashion with the catecholamines (for review see Reference 5). It seems that both the occupancy of the β-adrenergic receptor with agonists and the level of intracellular GTP determine the final output of cAMP by the enzyme adenylate cyclase. The role of GTP in the activation of adenylate cyclase has been extensively studied and will be discussed in detail when the mechanistic aspects of the mode of coupling of the receptor with the enzyme will be dwelled upon. Two other biochemical responses which are cAMP independent were claimed to be coupled to the β-receptor. One is the catecholamine-dependent Ca^{++} efflux, and the other is phosphatidylethanolamine carboxymethylation.

1. β-Receptor-Induced Ca^{++} Efflux

It was shown in both turkey[6] and human erythrocytes[7] that $^{45}Ca^{++}$ efflux is enhanced by β-agonists and blocked by β-antagonists. This effect is not mimicked by cAMP or dibutyryl cAMP and, therefore, is most probably not mediated by adenylate cyclase. It is interesting that β-receptor-dependent adenylate cyclase from turkey erythrocytes, as well as many other adenylate cyclases, are strongly inhibited by Ca^{++}. For the β-receptor-dependent turkey adenylate cyclase it was shown that the Ca^{++} ions interact at a specific allosteric site.[8,9] These findings suggest[6] that the first effect of a β-agonist is the deinhibition of the enzyme which is in the resting state in the inhibited Ca^{++} bound form, by releasing Ca^{++} from the regulatory site (Figure 3). These findings are so far restricted to the turkey and the human erythrocyte systems (which possess much fewer β-receptors), and it is not clear at this point whether the interaction between Ca^{++} and β-receptors is a general feature in the action of β-adrenergic receptors. Since Ca^{++} seems to be the second messenger of α-adrenergic response[10] and since it was found that α-agonists inhibit adenylate cyclase,[11-13] it is attractive to postulate that Ca^{++} functions as a regulatory link between α_1- and β-receptors. One difficulty with this finding is of course the fact that significant inhibition ocurs at the 10-0.1mm concentration range of Ca^{++}, when adenylate cyclase activity is assayed in membrane fragments.[8] It was observed, however, that incorporating the specific Ca^{++} ionophore A-23187 into the intact erythrocyte, in the absence of added free Ca^{++}, is sufficient to

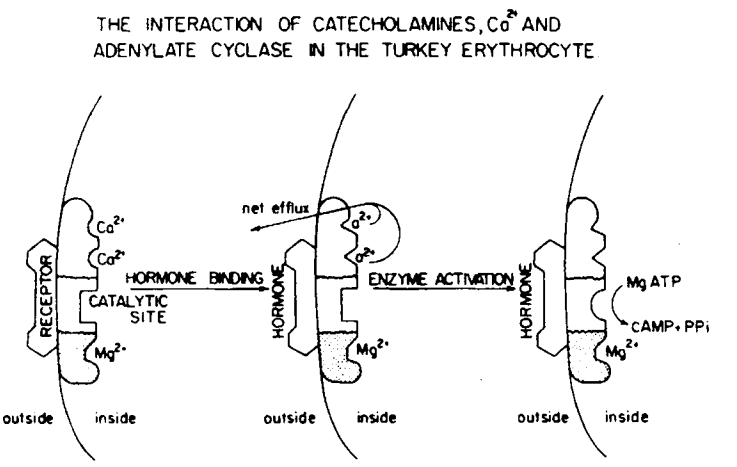


FIGURE 3. The control of adenylate cyclase by Ca^{++}: a suggestion. In this model it is postulated that the first event occurring upon β-agonist binding is the dissociation of Ca^{++} from its cyclase inhibitory site. The removal of Ca^{++} from the regulatory site is essential to bring the cyclase system to an activatable form. In the scheme it is shown that the enzyme system possesses at least two interacting calcium sites distinct from the Mg^{++} sites. The fact that the inhibitory Ca^{++} sites do not overlap with the Mg^{++} sites was shown by detailed kinetic analysis. Also, since the inhibitory effect of Ca^{++} possesses a Hill coefficient of 2, there are a minimum of two interacting sites.

induce 63% inhibition of the β-agonist-dependent activity in the intact cell.[6] This finding may be interpreted to mean that the materialization of the membrane-bound Ca^{++} is sufficient to induce enzyme inhibition, most probably by creating a high local high Ca^{++} concentration.

2. β-Adrenergic Receptor-Dependent Phospholipid Methylation

β-Adrenergic agonists were found to stimulate the enzymatic synthesis of phosphatidyl-*N*-mono methylethanolamine and phosphatidylcholine from phosphatidylethanolamine reticulocyte ghosts, in the presence of *S*-adenyosyl-L-methionine.[14] The stimulation is stereospecific, dose dependent, and inhibited by the β-blocker propranolol but not by α-adrenergic blockers. This β-receptor-dependent phospholipid methylation induces the increase of membrane fluidity with the concomitant increase in the β-receptor-dependent adenylate cyclase activity. The increase in the efficiency of the adenylate cyclase system may be due to the increased membrane fluidity which was found to play a key role in determining the efficiency of the coupling of β-receptors to adenylate cyclase in turkey erythrocytes. Similar results were recently reported for the β-adrenergic receptor-dependent adenylate cyclase from HeLa cells.[106] The β-receptor-dependent phospholipid methylation is not cAMP-dependent and it is not clear at present what role it plays in the generation of the eventual β-adrenergic response. It remains to be established whether the role of phospholipid methylation is solely to increase the efficiency of receptor-to-cyclase coupling due to the increase in membrane fluidity. Another unknown aspect of this phenomenon is of course the biochemical mechanism by which the receptor activates the phosphatidylethanolamine methylating enzyme(s). Recently, it was also observed that β-agonists induce the carboxymethylation of proteins in the rat parotid gland.[15] This effect is stereospecific for l-agonists, and is specifically blocked by β-blockers and not by α-adrenergic blockers. The role of β-adrenergic-dependent protein carboxymethylation in the parotid gland is not yet known. The time

course of protein methylation overlaps the time course of β-receptor-dependent α-amylase secretion, suggesting a relationship between protein methylation and the process of exocytosis.

B. α-Adrenergic Receptors

It has been demonstrated that the primary event occurring upon occupation of the α-adrenergic receptor by an α-agonist in the parotid gland is the influx of Ca^{++} which functions as the "second messenger".[10] Furthermore, the specific Ca^{++} ionophore A-23187, when incorporated into the cell membrane, can substitute for the α-adrenergic ligand and bypass the receptor-dependent mechanism. The influx of Ca^{++} as the primary event in the salivary gland (rat parotid) causes the efflux of K^+ ions with water (see Reference 10 and references therein). The efflux of potassium has also been recognized as an α-adrenergic response in guinea pig liver[16] and in adipose tissue.[17]

The stimulation of the pineal gland with l-epinephrine via the α-adrenergic receptor was found to be dependent on the presence of Ca^{++} in the incubation medium and results in a sevenfold increase in the cGMP level.[18] It seems from these studies that the influx of Ca^{++} is the first event induced by the α-agonist. The formation of cGMP seems to be the result of Ca^{++} influx. This is not surprising, as guanylate cyclase is a Ca^{++}-dependent enzyme. As is indicated in Section II, α-adrenergic receptors are involved in a variety of physiological activities, and it remains to be seen whether in each case Ca^{++} functions as the "second messenger". It has been claimed that in the central nervous sytem, α- as well as β-receptors are coupled to the enzyme adenylate cyclase. These observations were based on the observation (see Reference 19 and references therein) that α-adrenergic blockers inhibit the formation of cAMP, but no direct inhibition of adenylate cyclase by α-antagonists was demonstrated in these studies. In fact, α-agonists were shown to inhibit adenylate cyclase in membranes from platelets,[11] from neuroblastoma glioma hybrid cells,[12] and from fat cell membranes.[13] This inhibitory effect of α-agonists on the activity of adenylate cyclase requires the presence of GTP and Na^+ ions. It is not yet clear whether the α-receptor-linked GTP site is identical or different from the GTP stimulatory site known to be part of the eucaryotic adenylate cyclase system (see Section VIII.D). It is interesting to note that opiates and enkephalins were shown recently to induce the inhibition of PGE_1-dependent adenylate cyclase in neuroblastoma glioma hybrids where the inhibitory effect is GTP and Na^+ dependent.[20,21] Similarly, the inhibition of fat cell adenylate cyclase by the high-affinity inhibitory adenosine receptor requires the presence of GTP and Na^+ ions.[22]

Another biochemical response elicited by the activation of α-adrenergic receptors is the incorporation of inorganic $^{32}P_i$ into phosphatidylinositol in slices of the parotid gland.[23] This biochemical event was shown to be unrelated to the K^+ efflux and water secretion also induced by α-receptor activation. Interestingly enough, the divalent cation ionophore A-23187 which introduces Ca^{++} into the cell thus causing K^+ release,[10] has no significant effect on the incorporation of $^{32}P_i$ into phosphatidylinositol. Conversely, the α-receptor-induced phospholipid effect is maximal in the absence of Ca^{++} in the medium, when there is no K^+ release from the cell. In summary, it can be concluded that α-receptor activation leads to two independent biochemical events in the rat parotid gland: (1) an increase in membrane permeability toward extracellular Ca^{++} that enters the cell and causes K^+ release, and (2) an increased incorporation of $^{32}P_i$ into acidic phospholipids. So far the ability of α-agonists to inhibit adenylate cyclase activity in the rat parotid membranes has not been recognized, perhaps because the proper experimental conditions (GTP and Na^+) have not been met. It remains for future studies to determine whether all the α-adrenergic-induced effects result from a common mechanism.

It should be noted that the Ca^{++}-dependent K^+ release and the Ca^{++}-independent phospholipid effect were shown also to be induced by the activation of the muscarinic receptor in the same parotid gland preparation.[23] These latter observations tend to strengthen Selinger's assertion that the phospholipid effect and the Ca^{++}-dependent K^+ release are two independent biochemical responses to α-receptor stimulation, as well as to muscarinic stimulation. It seems that two classes of α-adrenergic receptors exist. One class α_1, is coupled to Ca^{++} influx and phosphotiolylinositol turnover, and another class, α_2, which is coupled to adenylate cyclose in an inhibitory mode. The action of the inhibitory α_2 receptors requires GTP, whereas the action of α_1 receptors does not require GTP. In Table 2 the main biochemical reactions coupled to catecholamine receptors are summarized.

C. The Relationship Between α-Receptors and β-Receptors

Almost every organ or tissue that possesses α-adrenergic receptors also possesses β-adrenergic receptors. The receptors elicit opposite physiological effects in the target organ (Table 1). Thus, it is possible that the final response of the organ in question depends on the relative activity of the two receptors. Since Ca^{++} functions as the second messenger of α-receptor action and inhibits the β-receptor-dependent adenylate cyclase, the latter may provide the link between α- and β-receptors in systems that possess both types of adrenergic receptors. α-Adrenergic receptors may actually function as negative regulators of adenylate cyclase when the latter is stimulated by receptors other than the β-receptors since it was shown that PGE_1-dependent cyclase is also inhibited by α-receptors (see previous section). It is interesting that Batzri et al.[24] find that in the rat parotid gland, the α-blocker phentolamine slows down the fall in the level of cAMP subsequent to epinephrine stimulation, as compared with a system in the absence of the α-blocker. This effect, however, may be due to secondary biochemical events other than the direct effect of Ca^{++} on the level of adenylate cyclase activity. For example, Ca^{++} is known to activate cAMP phosphodiesterases, and thus an increase in intracellular Ca^{++} may result not only in the inhibition of adenylate cyclase but also in the depletion of the cAMP pool. At this point it can only be stated that the interaction of α-receptors with β-receptors is not fully understood in biochemical terms and requires further investigation.

In the nervous system it was found that at catecholaminergic terminal where norepinephrine is released, α_2 and β_1 presynaptic receptors regulate the release of the norepinephrine neurotransmitter, irrespective of whether the postsynaptic receptor is of the β- or the α_1-type. It was found[2] that α-$_2$ autoreceptors induce the decrease of norepinephrine release, whereas β_1 presynaptic receptors enhance neurotransmitter release. This interrelation between the two types of adrenergic receptors seems to be a general feature of their interaction.

D. Interconversion of α- and β-Receptors?

Some reports in the literature have suggested that α- and β-receptors are two allosteric configurations of the same macromolecule. The experiments upon which this hypothesis is based were performed on frog heart, where it has been claimed that α-receptors prevail at low temperatures and are transformed into β-receptors at high temperatures. It was claimed (see Reference 25 and references therein) that stimulation of cardiac rate and contractibility by catecholamines has the properties of a classical β-adrenergic response when experiments are performed at warm temperatures (25 to 37°C) and of α-adrenergic response when experiments are performed at cold temperatures (5 to 15°C). Caron and Lefkowitz[26] examined this hypothesis by looking at the adenylate cyclase activity in a wide range of temperatures. These investigators exam-

Table 2
THE MAIN CLASSES OF CATECHOLAMINE RECEPTORS AND THEIR BIOCHEMICAL RESPONSES

Type of receptor	Ligand specificity	Specific blockers	Recognized biochemical responses
α	Norepinephrine > epinephrine > phenylephrine ⩾ isoproterenol	Phentolamine, ergotamine, phenoxybenzamine, dibenamine, yohimbine	Ca^{++} influx (α_1) Phosphatidylinositol turnover (α_1) Inhibition of adenylate cyclase (α_2)
β	Isoproterenol > epinephrine ⩾ norepineprhine ⩾ phenylephrine	Dichloroisoproterenol propranolol, alprenolol, pindolol, hydroxybenzylpindolol	cAMP formation Ca^{++} influx Phosphatidylethanolamine methylation
Dopamine	Dopamine > norepinephrine	Haloperidol	
		Chloropromazine, butaclamol Some α-blocking agents	cAMP formation*

Note: α- and β-adrenergic receptors are specific for the R stereoisomers (or the l-stereoisomer if the optical rotatory power is considered). The dopamine receptor is also stereospecific toward its ligands. This is reflected in the interaction of the postsynaptic dopamine receptor with (+) butaclamol which binds 100-fold more tightly than (−) butaclamol to the receptor.

* A secondary biochemical response other than cAMP formation is probably involved in the action of presynaptic dopamine receptors.

ined dog, rat, and frog heart and frog erythrocytes. In all of these cases it was found that the adenylate cyclase is stimulated by adrenergic ligands typical of β-receptors in a wide range of temperatures. Furthermore, the adrenergic inhibitors affecting cyclase in a wide range of temperatures were always of the β-type. α-Blockers had no effect on adenylate cyclase over a wide range of temperatures. As Caron and Lefkowitz[26] pointed out, the studies claiming the α to β interconversion were performed on an intact tissue,[25] whereas the adenylate cyclase measurements were performed on membrane fragments.[26] Thus, it still remains possible that the interconversion of α- and β-receptors requires the intact cellular structure. The integrity of the cellular structure may preserve the biochemical mechanism which may be responsible for α- to β-receptor interconversion.

In conclusion, the possibility of α- to β-receptor interconversion still remains in light of the pharmacological experiments, although it does not at present find any support from direct biochemical experiments. Obviously, structural data on the β- and α- receptors become extremely critical, if one wishes to explore more directly the biochemical correlations between these two types of receptors.

E. Dopamine Receptors

Dopamine, similar to epinephrine and norepinephrine, is a neurotransmitter in the central nervous system and in some peripheral tissues. In some areas of the brain it was demonstrated that the dopamine receptor is coupled to adenylate cyclase (see Reference 3 and references therein). The dopamine receptor is different from the β-adrenergic receptor in its ligand specificity and in its response to specific blockers (Figure 2). Thus, dopamine receptors coupled to adenylate cyclase respond to dopamine better than to norepinephrine (Table 2), whereas in β-receptors the order of potency is reversed. β-Adrenergic blockers such as propranolol do not affect dopamine-dependent

adenylate cyclase, whereas phenothiazine-type compounds such as chloropromazine and butyrophenones such as haloperidol act as specific blockers of dopamine-dependent adenylate cyclase (Table 2) and have no effect on β-adrenergic receptor-dependent adenylate cyclase. Whether dopamine receptors are coupled to other biochemical signals such as ion fluxes is not yet clear. In neuroblastoma, α-adrenergic blockers were found to affect dopamine-dependent adenylate cyclase, whereas β-adrenergic blockers were found to have an effect only at very high concentrations.[27] Similarly, dopamine-sensitive adenylate cyclase from the mesolimbic system was found to be inhibited by α-blockers but not at all by β-blockers.[28] Thus, there is some overlap in ligand specificity of the dopamine receptor with the α-adrenergic receptor. Furthermore, binding studies using ^{3}H-d-LSD have shown [29]that the latter compound monitors dopamine receptors in the brain. One should remember, however, that other lysergic acid derivatives such as ergocryptine were claimed to be specific α-adrenergic blockers.[30] The situation is further complicated by the fact that d-LSD is a well-known serotonin antagonist in several smooth muscle systems and a weak mixed agonist at postsynaptic serotonin receptors in the brain. Data are also available to show that d-LSD interacts with presynaptic receptors on serotonin neurons which are distinct from the postsynaptic serotonin receptor sites. The partial overlap among dopamine receptors, α-adrenergic receptors, and serotonin receptors makes the biochemical characterization of dopamine receptors more difficult. Recently, the existence of multiple forms of dopamine receptors has been recognized (see Reference 3 and references therein). It is apparent that only through careful binding experiments using a *variety of ligands in conjunction with biochemical experiments* such as the determination of the effect of the different drugs on dopamine-dependent adenylate cyclase, can one approach the true characterization of the dopamine receptors.

IV. RADIOLIGAND BINDING ASSAY OF β-ADRENERGIC RECEPTORS

In 1974, it was firmly established that catecholamine binding assays do not monitor the authentic β-adrenergic receptors for two reasons.[31,32] First, the receptor concentration accessible experimentally (up to 10 n*M*) is far below the catecholamine-receptor dissociation constant (100 n*M* to 100 μ*M*). Also, because of the high dissociation constants, the filter assay cannot work anyway since the lifetime of the receptor ligand complex is much shorter than the fastest experimentally available filtration time. Second, catecholamines bind to many nonreceptor components in the membrane preparations studied, and thus the nonspecific catecholamine binding constitutes over 90% of the measured catecholamine bound.[31,32] Also in 1974, radioactively labeled β-adrenergic blockers were found to monitor reliably β-adrenergic receptors. The first ligand used was ^{3}H-propranolol;[31,33] shortly thereafter and independently, ^{125}I-hydroxybenzylpindolol[34] and ^{3}H-dihydroalprenolol[35] (reviewed in Reference 36) were introduced as specific ligands for the radioassay of β-adrenergic receptors. The use of these radioactively labeled ligands has since become a routine procedure to monitor β-adrenergic receptors in a large variety of cells. The affinity of these β-blockers to the β-receptors is very high, being three to six orders of magnitude higher than the affinity of β-agonists toward the β-receptors (Table 3) — namely, below the nanomolar range. Therefore, with these compounds one can monitor low concentrations of these receptors. The binding of these compounds to the β-receptor is stereospecific for the R stereoisomer, and therefore the R-antagonists are displaced from the β-receptor specifically by R(−) catecholamines and much less effectively by S(+) catecholamines. S(+) catecholamines are usually about 100-fold less effective in displacing the R-antagonist from

Table 3
THE AFFINITY OF DIFFERENT β-BLOCKERS TOWARD THE β-RECEPTORS

β-Blocker	$K_{Diss.}$ (nM) From kinetics*	From binding experiments
(−) Propranolol	1.3 ± 0.1	1.2 ± 0.1
(−) Alprenolol	2.0 to 10 ± 1	2.0 to 10 ± 1
(−) Iodohydroxybenzylpindolol	—	0.02 ± 0.002 to 0.5 ± 0.2

Note: Data are taken from references cited in the text. The values quoted represent the range of constants reported between 1974 and 1979.

* Competitive inhibition of (−) catecholamines in the adenylate cyclase reaction.

the β-receptor. It is not clear, however, whether S(+) catecholamines bind to β-receptors with 100-fold less affinity or the S(+) catecholamines are actually completely ineffective but contaminated with the respective R(−) catecholamine. The dissociation constants found for these β-blockers using binding experiments match very closely the inhibition constants found from their competitive inhibition with catecholamines in the assay of the β-receptor-dependent adenylate cyclase reaction.

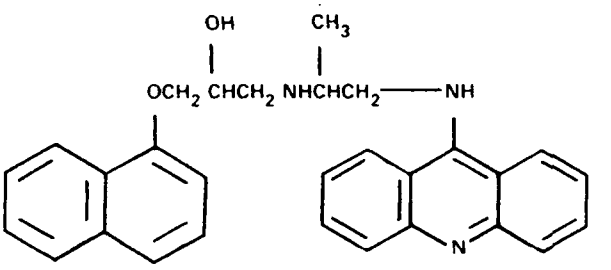
Radioactively labeled blockers can, in principle, be used to monitor also detergent-solubilized receptors, provided the receptor does not denature in the process of solubilization. Such attempts have recently been reported, where digitonin-solubilized β-adrenergic receptors from frog and turkey erythrocyte membranes were monitored using ^{3}H-alprenolol[37] and ^{125}I-hydroxybenzylpindolol, respectively.[106] Since ^{125} I-hydroxybenzylpindolol exhibits extremely high affinity toward β-adrenergic receptors, it can be used to monitor these receptors in intact cells.[38]

Recently, the high affinity agonist ^{3}H-hydroxybenzylisoproterenol was used successfully to monitor β-adrenergic receptors.[39] The availability of specific ligands to monitor β-adrenergic receptors has also made it possible to monitor receptor cryptization due to desensitization. Using radiolabeled β-blockers, it can be demonstrated that β-receptor desensitization (down regulation) induced by catecholamines is manifested by both a decrease in the number of β-adrenergic receptors and in the β-receptor-dependent adenylate cyclase activity (see Section IX.A).

V. THE STUDY OF β-RECEPTOR DISTRIBUTION ON CELL SURFACES

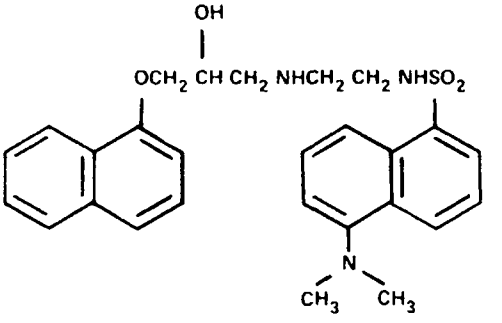
Two main approaches to study the distribution of β-receptors on cell surfaces have been developed in recent years. One is based on the use of fluorescent β-blockers and the other a β-blocker that possesses an ultrastructural anchor for ferritin attachment.

Two fluorescent β-blockers, 9-AAP and DAPN,[40] and NBD-propranolol (Figure 4) proved to be useful in probing β-receptors in vivo and in vitro. These compounds were shown to bind in a stereospecific manner to β-adrenergic receptors in vivo upon their injection into rats and mice. Both peripheral β-receptors in the central nervous system bind these fluorescent antagonists and become visible in the fluorescent microscope. Prior injection of l-propranolol, but not of d-propranolol, into the animal prevents the binding of the fluorescent blocker, and therefore the appearance of the characteristic fluorescence pattern.[41] Furthermore, treatment of the rat or the mouse with 6-



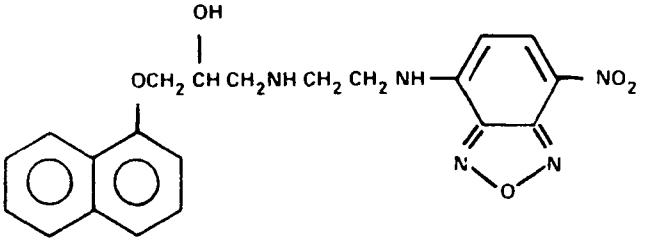
9-AMINOACRIDINO PROPANOLOL

(9–AAP)



DANSYL ANALOGUE OF PROPRANOLOL

(DAPN)



7–NITROBENZ-2–OXA–1,3 DIAZOLE ANALOGUE OF PROPRANOLOL

(NBD–PROPRANOLOL)

FIGURE 4. Fluorescent β-adrenergic blockers.

hydroxydopamine, which causes the disappearance of the catecholamine storage vesicles in the presynaptic region, does not prevent the appearance of the fluorescence pattern in the Purkinje cell layer upon injection of 9-AAP[42] or of DAPN.[107] These latter results indicate that the localization of the fluorescent compound is, indeed, in the postsynaptic β-receptors.

The binding of 9-AAP can be monitored directly by the fluorometer.[40] One difficulty in the use of 9-AAP and DAPN is that the two compounds emit fluorescence at an identical fluorescence range as tissue "autofluorescence", and thus the distinction between autofluorescence and DAPN or 9-AAP fluorescence may become sometimes cumbersome. Therefore an attempt is currently being made[107] to develop fluorescent β-blockers which fluoresce at wavelengths well above the "yellowish" region. Such a compound is NBD-propranolol (Figure 4). The fluorescence spectrum of NBD-propranolol is red and it was found to be a potent β-blocker, as measured by its ability to

inhibit competitively β-receptor-dependent adenylate cyclase and the binding of ^{125}I-hydroxybenzylpindolol (K_d = 1 to 2 × 10^{-8} *M*).[43] It remains to be seen whether this compound is suitable for studies in vivo.

The use of specific ligands to probe catecholamine receptors is complementary to the formaldehyde method of Falck et al.[44] and of the glyoxylic acid method[45] for the mapping of catecholaminergic pathways. These latter techniques do not discriminate between the different types of catecholaminergic neurons, as all catecholamines condense with formaldehyde and glyoxylic acid to yield a fluorescent derivative. Serotonin also yields a fluorescent derivative upon condensation with formaldehyde or glyoxylic acid, and therefore serotonergic pathways become visible in the fluorescence microscope. The development of specific fluorescent ligands for each type of receptor may become a powerful tool for the mapping of the different types of catecholaminergic receptors. Obviously, this approach can be extended for the study of other neurotransmitter and hormone receptors once the suitable probes are prepared.

The availability of fluorescent agonists or antagonists makes it possible also to study directly the receptor in vitro, if high enough concentrations can be generated in the test tube. Preliminary experiments[40] using 9-AAP indeed demonstrate that its binding to the β-adrenergic receptor in turkey erythrocyte membranes can be monitored in vitro. It is hoped that such compounds will be a useful tool to study receptor activity, once relatively high concentrations of catecholamine receptors become a laboratory reality.

Little is known about the topographical relationship between three recognized components of the β-receptor-linked adenylate cyclase; namely, the β-adrenergic receptor, the catalytic moiety of adenylate cyclase, and the guanyl nucleotide binding protein. Recently it was shown that the β-receptors can be transferred from one cell to another upon virus-induced cell fusion[46] or polyethyleneglycol-induced fusion.[47] This finding, however, does not preclude the possibility that the receptor and the enzyme are separated from each other by relatively large distances in the native situation and that the interaction between the enzyme and the receptor is bimolecular. Recent evidence[48,49] indicates that the receptor constantly moves in the membrane matrix of the turkey erthyrocyte and that the enzyme is activated during the collision with the agonist receptor complex (see Section VIII). However, it is not clear yet whether this is the mechanism of cyclase activation in other cells. Also, there is no clue as to whether the enzyme and the receptor are confined to domains on the cell membrane or move freely over the entire surface of the cell membrane. For hormones such as insulin and epidermal growth factor, it was shown that subsequent to hormone binding the receptor-ligand complexes collect over "coated pits" and are then internalized.[50] This, however, was not demonstrated so far for low molecular weight hormones.

More recently,[51] through the use of the ultrastructural probe biotin propranolol (Figure 5), it was shown that β-receptors in skeletal rat muscle cells grown in culture (L6P cells) are mainly localized in the region of microvilli. The technique used was as follows: subsequent to the application of the potent β-blocker, biotin propranolol, to the cells, an avidin-ferritin conjugate was applied such that the location of β-receptors becomes visualized in the electron microscope. Again, it is not clear whether the binding event triggers the clustering ("patching") or whether the receptors in their unbound state are preclustered in these domains. It is interesting to note that "coated pits" are localized at the base of microvilli.[52] More experiments of this nature must be performed in order to obtain a better idea about the generality of this finding.

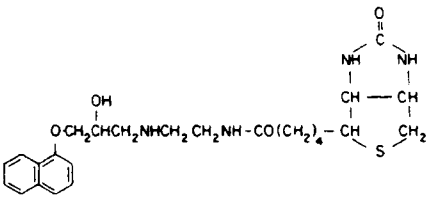


FIGURE 5. Biotin analogue of propranolol. This compound was used to localize β-receptors on muscle cells grown in culture. Subsequent to application of the compound, the cells were fixed and exposed to a ferritin-avidin conjugate. β-Receptors became visible as ferritin dots.

VI. AFFINITY LABELING OF THE β-ADRENERGIC RECEPTOR

Affinity labeling of the β-adrenergic receptor has recently been achieved by using a reversible β-blocker to which the reactive group bromoacetyl was attached.[53,54] The compound *N*-(2-hydroxy-3-naphthyloxypropyl:-*N'*-bromoacetyl-ethylenediamine (Figure 6) has been shown to inhibit irreversibly the epinephrine-dependent adenylate cyclase activity without damaging the F^--dependent activity in turkey erythryrocyte membranes.[53,54] Furthermore, propranolol and l-epinephrine offer protection against the affinity labeling reaction. Similarly, the compound was shown to inhibit irreversibly the hormone-stimulated activity in a whole turkey red cell.[54] The loss of epinephrine-dependent activity is accompanied by the loss of ^{3}H-propranolol binding[54] and of ^{125}I-iodohydroxybenzylpindolol binding,[48] demonstrating directly the loss of β-receptor after treatment with the affinity label. More recently, the ^{3}H-affinity label was synthesized,[55] and attempts are being made to characterize the protein moiety of the β-receptor. The tritium-labeled affinity label was reacted with intact L6P cells and with intact turkey erythrocytes. The treatment of the two types of cells with the affinity label was conducted in the presence and in the absence of the potent β-adrenergic blocker hydroxybenzylpindolol. As it was shown earlier,[53,54] β-blockers and β-agonists protect against the labeling of the β-adrenergic receptor. When the membranes of the labeled turkey erythrocytes and L6P cells were solubilized and subjected to gel electrophoresis in the presence of sodium dodecyl sulfate, two tritium-labeled protein bands could be identified: 37,000 and 41,000 in molecular weight. The labeling of these two bands is significantly diminished when they are exposed to the ^{3}H-affinity label in the presence of the high affinity β-blocker, hydroxybenzylpindolol ($K_D \sim 1.0 \times 10^{-10} M$). From these experiments it was concluded that these two bands probably represent subunits of the β-adrenergic receptor. Another encouraging observation was that the protein bands which became radioactively labeled constitute only a small fraction of the total membrane protein and are barely detected by the protein stain.[55] In view of the observation by Hagar and colleagues[56] and by Limbrid and Lefkowitz[57] that the detergent-solubilized β-receptor has a molecular weight of 75,000 or 130,000 to 150,000 or higher, respectively, it seems that the β-adrenergic receptor is an oligomer with a molecular weight of around 40,000. It was also pointed out that proteins of indistinguishable electrophoretic mobility become labeled with the affinity label in L6P cells and in turkey erythrocytes.[55] This observation suggests that the structure of the β-adrenergic receptor is similar in different types of cells and may have been conserved during evolution. Recently, the synthesis of an effective β-receptor directed photo-affinity label, similar in structure to the bromoacetyl derivative, was reported.[58]

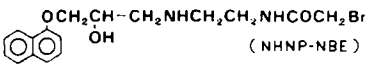


FIGURE 6. A β-receptor directed affinity label.

In a recent report,[59] it was claimed that β-adrenergic receptors mediating the relaxation of the guinea pig *Taenia coli* were photo-affinity labeled with l-isoproterenol or 2-(2-hydroxy-3-isopropylamino-propoxy)-iodobenzene. The irradiation of the guinea pig *T. coli* in the presence of either compound resulted in the irreversible loss of response to β-adrenergic agonists such as l-isoproterenol. Wrenn and Haber[60] reported recently about the preparation of an antibody against the β-receptor. Rabbits were immunized with a deoxycholate-solubilized fraction from dog heart and the serum isolated from these rabbits was found to inhibit specific ^{3}H-propranolol binding as well as the l-isoproterenol-dependent adenylate cyclase activity in dog heart.

VII. SOLUBILIZATION AND PURIFICATION OF THE β-ADRENERGIC RECEPTOR

As mentioned above, attempts are being made to solubilize and purify the β-adrenergic receptor. However, the absolute amount of β-receptor obtained from turkey erythrocytes, frog erythrocytes, or S49 lymphoma cells is extremely small. The digitonin-solubilized receptor is reported to preserve its ligand binding ability by two groups,[37,57] but another group encountered difficulties when using digitonin.[56] This latter group chose Lubrol-PX for the solubilization of the β-adrenergic receptor. Lubrol-PX-solubilized β-receptor from S49 lymphoma cells was reported to bind ^{125}I-hydroxybenzylpindolol when added prior to solubilization, whereas the solubilized receptor was unable to bind ^{125} I-hydroxybenzylpindolol subsequent to solubilization.[56] Recently, successful attempts in partial purification of the solubilized β-receptor have been reported. In both cases[37,61] an affinity column based on (−)alprenolol was prepared and used to purify the β-adrenergic receptor a few thousand-fold. It was found that the ligand specificity and stereospecificity of the receptor were preserved in the solubilized state. More work is obviously required before relatively pure β-receptor will be available in quantities sufficient to perform biochemical studies.

VIII. COUPLING BETWEEN THE β-RECEPTOR AND ADENYLATE CYCLASE

A. Structural Aspects

A number of possibilities with respect to the mode of interaction between the receptor unit and the cyclase unit were considered.[48] These possibilities can be summarized as follows:

1. The receptor site and the active site of the adenylate cyclase reside on the same protein where the receptor faces the outside of the cell and the active site faces the inner surface of the membrane. Such a situation would be analogous to the adenylate cyclase from *Brevobacterium liquifaciens* which was found to be a dimer composed of two identical subunits in which both the active site and the regulatory site for pyruvic acid reside. This model was favored by certain workers, but is unlikely to apply to the eucaryotic enzyme. A large variety of data suggest that the receptor and the catalytic moiety are separate protein units, products of different genes.

2. The receptor and the enzyme occur as an oligomeric complex R_nE_m (R = receptor, E = enzyme) composed of two distinct subunits in analogy to aspartate transcarbamylase. The complex is composed of n receptor subunits and m catalytic subunits. This type of model was favored by Robison and co-workers.[62] To update this model one might include a third component — namely, the GTP regulatory unit — which is an integral part of the cyclase system. Thus it will be $R_nG_mC_l$ where the stoichiometry of the three components is n:m:l. We shall define the complex CG as E.
3. The possibility exists that a physical separation between R and E(E = CG), concomitant with enzyme activation, takes place upon hormone binding. This model is reminiscent of the cAMP-dependent protein kinase and was suggested as a possibility by some workers.
4. The receptor and the enzyme are physically separated, and the hormone receptor and the enzyme are moving randomly, relative to each other in the lipid bilayer. In the absence of agonist, the affinity of the enzyme to the receptor is negligible, and an encounter between the two entities does not yield any activation. Upon agonist binding, a structural change occurs in the receptor, which allows a fruitful encounter between the agonist-bound receptor and the enzyme. During this encounter the enzyme becomes activated. It may even be that affinity between the two entities will increase in the presence of agonist and, thus, a ternary complex will become stabilized.

In early 1976 we stated,[63] "So far no experimental evidence is available to distinguish between these four possibilities in terms of their closeness to the truth, although we feel it is evident that alternative (1) can be discarded." Today, 3 years later, we are much wiser on this subject due to the efforts of many laboratories. Alternative (1) can now be almost safely rejected, as three lines of evidence indicate strongly that the agonist recognition site — the receptor and the catalytic site of adenylate cyclase — resides on two separate macromolecules. First, attempts to solubilize and to purify[37,56,57] the β-adrenergic receptor led to the claim that this receptor can be easily separated from the catalytic component of adenylate cyclase. These studies are in line with the basic assumption that the receptor and the enzyme present two separate macromolecules. Second, elegant experiments recently performed by Orly and Schramm[46] have provided another proof of this hypothesis. In these experiments turkey erythrocytes, in which the catalytic activity had been inactivated by *N*-ethylmaleimide or by heat, served to contribute the β-adrenergic receptor. Friend erythroleukemic cells (F cells), which possess no β-adrenergic receptors, served to contribute the enzyme adenylate cyclase. The erythrocytes in which the enzyme had been inactivated were fused with the F cells using *Sendai* virus. The cell ghosts of the fused preparation demonstrated l-isoproterenol-dependent adenylate cyclase. These experiments reveal, therefore, that the β-adrenergic receptor of the turkey erythrocyte must have become functionally coupled to adenylate cyclase of the mouse F cells. More recently solubilized β-receptor from turkey erythrocyte was added to β-receptor-deficient Friend erythroleukemia cells and generated a β-adrenergic responsive cyclase.[47] Third, fractionation of membranes from frog erythrocytes[64] reveals that the catalytic moiety and the β-adrenergic receptors are not equally distributed. This finding corroborates the idea that the β-receptors and the enzyme are not contiguous. Thus, coupling must have occurred between the preexisting components. Fourth, recent experiments (see Reference 65 and references therein) on clones of lymphoma cells demonstrate that the GTP binding protein is a product of a separate gene. It is possible to obtain lymphoma S49 cells which possess normal β-adrenergic receptor and the catalytic moiety of adenylate

cyclase. In these mutants the enzyme cannot be stimulated by β-agonists but only by Mn^{++} which acts directly on the catalytic moiety of the enzyme. The enzyme, however, cannot be stimulated by NaF or by GppNHp because of the presence of a defective GTP binding protein. Membranes from this mutant cell, termed AC^-, can regenerate the normal β-receptor-dependent cyclase activity once a GTP binding protein from the wild type cell is added back in the presence of detergent. This reconstitution of catecholamine-sensitive cyclase establishes the essential role of the GTP regulatory unit.

In summary, although none of the components of a hormone-responsive adenylate cyclase has been purified, it is quite clear that three components are involved: (1) the receptor (R) which faces the outside of the cell and which interacts with the hormone or the neurotransmitter, (2) the catalytic moiety (C) responsible for the conversion of ATP to cAMP, and (3) the GTP regulatory units (G). The two latter entities face the cytoplasm. It seems that the simultaneous occupancy of the receptor by agonist and of the regulatory unit by GTP is required to bring about enzyme activation. The exact stoichiometry and disposition of these units are not yet known and should be the subject of active investigation.

B. Mechanistic Aspects

Over the past few years it has become apparent that the receptor and the catalytic moiety are not the only components of the receptor-cyclase complex. Mainly through the pioneering studies of Rodbell and colleagues,[66] it became apparent that a third component, the transducer, plays a decisive role in the processing of the hormonal signal. It turns out that the binding of the hormone or of the neurotransmitter is a necessary event for the activation of adenylate cyclase, but is not sufficient (see References 67 and 68 and references therein). The nucleotide GTP *must* be present so that the hormone-induced activation of adenylate cyclase will take place. The role of the GTP regulatory unit was not recognized for a long time, probably because the substrate ATP used in the cyclase assay is usually contaminated with enough GTP to saturate the GTP regulatory site that binds the nucleotide with an affinity constant in the micromolar range. Indeed, when ATP free of GTP is used in the cyclase assay, the dependence of the cyclase activity on GTP can be demonstrated.

It is now generally accepted that GTP functions as an intracellular regulator which interacts with a specific regulatory site on the receptor-cyclase system and activates the enzyme with the hormone in a synergistic manner. It was also found to be generally true that GTP analogues such as GppNHp, GTPγS, and $GppCH_2p$ (See Reference 68 and references therein) activate the hormone-dependent adenylate cyclase in a quasi-irreversible fashion and, in the presence of hormone, induce the formation of a highly active and extremely stable adenylate cyclase. Detailed kinetic analysis on the β-adrenergic receptor-dependent adenylate cyclases[48,67,68] reveal that the role of the agonist is to facilitate the activation of the adenylate cyclase by the guanyl nucleotide. The efficiency of the β-receptor directed ligand diminishes progressively where: l-epinephrine = l-isoproterenol = l-norepinephrine > dopamine > l-phenylephrine > l-isoterenol > metanephrine. That is, the lower the efficacy of the agonist, the less efficient is the process of the ligand-induced cyclase activation.[69] Pure antagonists such as l-propranolol have no effect on the rate of cyclase activation. The extent of enzyme activation by the guanyl nucleotide alone (basal activity) varies from system to system, but in every case the agonist facilitates the activation process. In our notation below we depict the cyclase unit as E where E includes the GTP regulatory unit. Independent evidence indicates that the E and the GTP unit are associated with each other even in the detergent-solubilized state.[70]

C. Kinetic Aspects of the GTP-Hormone Interrelation

The activation of adenylate cyclase to its activated state requires the simultaneous binding of the agonist and of the guanyl nucleotide to their respective sites. When both sites are occupied, the enzyme is converted from its inactive E state to its activated state E′. Termination of the hormonal signal occurs concomitantly with the hydrolysis of GTP at the guanyl nucleotide regulatory site to GDP and P_i. Indeed, in turkey erythrocytes[71] and in pigeon erthrocytes,[109] the β-receptor-dependent GTPase activity can be measured directly. The hydrolysis step per se is independent of the continued presence of the agonist at the receptor site.[48,67,68] The enzyme cannot, however, be reactivated until a new molecule of GTP binds to the guanyl nucleotide regulatory site, a process which can occur *only* if the receptor is occupied by an agonist and the GDP produced in the GTPase step is removed from the GTP regulatory site.

It has been suggested[72,73] that the rate-limiting step in hormone-induced cyclase activation is the dissociation of GDP from the GTP regulatory site and that the role of the hormone is to facilitate this step. It is therefore possible that basal activity reflects the hormone-independent rate of GDP dissociation from the GTP regulatory site. It follows that the different basal activities exhibited by different cyclase systems reflect different rates of hormone-independent GDP dissociation. In the presence of nonhydrolyzable GTP the latter remains tightly bound at the guanyl nucleotide site, the hydrolytic step does not take place (see References 67 and 68 and references therein), and the enzyme remains in its active state E′. In the presence of hormone and GTP, the only two species of cyclase in the system are E, the inactive form of the enzyme, and the cAMP producing form E′. Once steady state has been reached, the system can be described by the following equation:[67]

$$E \underset{k_{off}}{\overset{\text{GTP, agonist, } k_{on}}{\rightleftharpoons}} E' \qquad (2)$$

Thus, the total enzyme concentration in the membrane $[E_T]$ under these conditions is given by:

$$[E_T] = [E] + [E'] \qquad (3)$$

Applying the steady-state conditions:

$$k_{on}[E] = k_{off}[E'] \qquad (4)$$

Inserting (3) into (4), one obtains:[67]

$$[E'] = \frac{[E_T]}{1 + \frac{k_{off}}{k_{on}}} \qquad (5)$$

Namely, only a fraction of the total cyclase pool is in its active form. The fraction $[E']/[E_T]$ can in fact be measured directly by determining the ratio of the maximal specific activity in the presence of GTP to that in the presence of GppNHp. In the latter situation, all of the enzyme pool is converted to the active form and thus the specific activity is given by the term "kcat $[E_T]$" where kcat is the turnover member of the cyclase system. In the presence of GTP, however, the maximal specific activity is given by the term "kcat [E′]". It follows that the ratio between the two specific

activities yields directly by the ratio $[E']/[E_T]$. According to the two state models derived here, the ratio $[E']/[E_T]$ is determined by the ratio k_{off}/k_{on}. The kinetic constant k_{on} depends on hormone concentration and its maximal value is attained at saturating agonist concentration. It is apparent from Equation 5 that the level of cyclase activity increases as k_{on} increases. The first order rate constant k_{off}, depicting the decay of the activated state to its inactive form, represents the GTPase step at the regulatory site. For the turkey erythrocyte β-adrenergic-dependent adenylate cyclase, the values of k_{on}[48,73] and k_{off}[48,69,71] were measured directly. By following the rate of adenylate cyclase activation by saturating concentrations of l-epinephrine and saturating concentrations of GppNHp,[48,73] k_{on} was determined. Under these conditions, $k_{off} = 0$, since GppNHp cannot be hydrolyzed, and thus *all* the cyclase molecules are converted to the activated form of the enzyme, as was indeed found — namely, $[E'[= [E_T]$. This value was found to be in the range of $k_{on} = 0.4$ to 1.0 min^1 at 37°C. The value of k_{off} was also measured directly by two independent methods for the turkey erythrocyte system. One method involves the measurement of the rate constant of GTP hydrolysis at the regulatory site by the GTPase assay which was recently developed.[71] The second method involves the measurement of the rate decay of the activated state E′ to its inactive form by a quenching experiment[48,69] carried out as follows: The enzyme is incubated with hormone, GTP, and nonradioactive ATP; at zero time an excess of antagonist and $\alpha[^{32}P]$ATP are added simultaneously. At this time E cannot be reconverted to E′ as this conversion requires the continued presence of agonist at the receptor site and E′ decays to E with the characteristic k_{off}. Concomitantly with this decay [^{32}P] cAMP is continued to be produced by the vanishing activated form of the enzyme E′. The time course of [^{32}P]cAMP formation reflects the time course of the decay of E′ to E. The characteristic rate constant of this process is k_{off} and thus can be easily obtained. When an antagonist is not available, the quenching can be performed by GDPβS.[74] The latter replaces GDP at the regulatory site, subsequent to its formation, and because of its tight binding, prevents the binding of GTP and thus blocks reativation of the enzyme. It must be emphasized that these methods can be applied to *any* adenylate cyclase system, whereas the success of the GTPase assay depends substantially on the ability to measure the specific hormone-dependent GTPase activity over the high background nonspecific nucleotide triphosphatase activity. Equation 5 can be rearranged to:

$$\frac{[E_T]}{[E']} = 1 + \frac{k_{off}}{k_{on}} \qquad (6)$$

and one can examine whether the two-state model discussed here for the cyclase system is indeed applicable. This can be done as follows: k_{off} and k_{on} can be measured independently and so is the ratio $[E_T]/[E']$. The latter ratio represents the ratio of the specific activity in the presence of GppNHp to that in the presence of GTP. One can then examine whether indeed the ratio $[E_T]/[E']$ and $k_{off}/n\ k_{on}$ are related by Equation 6.

Such a comparison has in fact been carried out for the turkey erythrocyte β-receptor system, and the correspondence was found to be excellent.[67,69] This finding supports the view that the two-state model for the cyclase system accounts well for the experimental data. The simple model described in Equations 2 through 5 suggests also a mechanistic approach to the study of the nature of partial agonism in the cyclase system. Partial agonists induce a smaller fraction of the cyclase to be converted into its active form E′, thus yielding a lower specific activity as compared with full agonists. This may result either from a lower k_{on} value or a higher k_{off} value (or both). This was recently examined in detail in the case of turkey erythrocyte β-receptor-dependent

adenylate cyclase. It was found that k_{off} is identical for nine full and partial agonists, whereas k_{on} is the parameter which is agonist-dependent.[69] Since it was also found in the latter case that $k_{off} \gg k^{on}$, it became apparent from Equation 5 or 6 that the level of cyclase activation, E', is linearly dependent on k_{on}. Indeed, when the steady-state level of cyclase activity is plotted against k_{on} for nine different agonists, a linear relationship is obtained.[69] This finding corroborates further the claim that it is sufficient to consider a two-state model for the cyclase system. Another recent finding (see also below) is that the cholera toxin-induced ADP-ribosylation of the GTP binding protein is the origin of the increase in the activity of adenylate cyclase (E' according to our notation). It can be shown[75] that this covalent modification results in the decrease of the GTPase step (k_{off}) and thus yields an increase in the steady-state level of active cyclase in the presence of GTP. These data[75] lend further support to the model of cyclase activation discussed above.

D. The GTP Binding Protein

The hypothesis that the guanyl nucleotide regulatory site represents a separate regulatory unit was recently verified directly. By exposing Lubrol-PX-solubilized pigeon erythrocyte membranes to a GTP-sepharose matrix, GTP binding proteins can be separated from the cyclase catalytic unit.[70,76] These proteins can then be dissociated from the GTP-matrix by GppNHp or GTP and, upon their addition to the cyclase which was deprived of the guanyl nucleotide binding proteins (G-protein), reconstitution occurs and adenylate cyclase activity is regenerated in the presence of GppNHp.[76,77] Furthermore, Pfeuffer has found that the guanyl nucleotide binding protein isolated from pigeon erythrocytes is also capable of activating rabbit myocardial adenylate cyclase which was previously depleted from GTP binding proteins.[70,76] This latter finding strongly indicates that this regulatory protein is a *universal* unit of the adenylate cyclase system and, therefore, can couple with a catalytic unit from many species. Affinity labeling experiments,[76] using a GTP derivative, indicate that probably only one of the GTP binding proteins — possessing a molecular weight of approximately 42,000 — which binds to the GTP-matrix is the guanyl nucleotide regulatory unit which is strongly attached to cyclase. More recently (see below) it was found that it is the same 42,000 GTP binding subunit that is ADP ribosylated when the pigeon erythrocyte membrane is exposed to cholera toxin. It remains to be established whether the GTP binding protein responsible for cyclase activation possesses the GTPase activity. The fact that ADP ribosylation inhibits the β-receptor-dependent GTPase activity in turkey erythrocytes supports this assumption.

The presence of the GTP binding protein is essential also for the reconstitution of fluoride activity (see Reference 65 and references therein). That is, the ability of NaF to stimulate adenylate cyclase requires the association of the catalytic moiety with the GTP binding protein. This is the reason why this protein has also been termed the G/F protein. Membranes from mutant S49 cells possessing β-adrenergic receptors but lacking hormone-dependent cyclase activity (AC^-) were shown to regain hormone responsiveness upon addition of the G/F protein extracted from wild-type cells.[65] It seems therefore that the stable association of the GTP binding protein with the adenylate cyclase moiety does not require penetration of the protein into the membrane. This assertion is based on the findings[65] that (1) the GTP binding protein can be removed from the membranes and solubilized using low urea concentrations or EDTA, and that (2) the protein seems to bind little detergent as contrasted with the β-receptor.[65] Upon solubilization of β-receptor-dependent adenylate cyclase, the receptor (R) separates easily[56] from the cyclase (C), whereas the GTP regulatory unit (G) tends to associate with the catalytic unit C.[70] In fact, these findings are not in contradiction

with the assumption that the receptor does not form a stable complex with the cyclase complex altogether. In the turkey erythrocyte system there is independent evidence to support the latter situation (see below).

E. The Mode of Action of Cholera Toxin

It has been shown that a variety of cells and membranes derived from these cells exhibit an increased adenylate cyclase activity subsequent to treatment with cholera toxin in the presence of NAD^+. It was suggested[75] and, indeed, demonstrated that cholera toxin causes a *decrease* in the GTPase step,[75] namely, in k_{off} (Equations 5 and 6), thus increasing the steady-state concentration of the activated form of adenylate cyclase in the presence of hormone and GTP. The cholera toxin-induced activation of the adenylate cyclase system is most probably due to the ADP-ribosylation[75,78-80] of the 42,000 mol wt guanyl nucleotide binding protein. The inhibition of the GTPase step is responsible for the observation that adenylate cyclase activation by GTP, subsequent to cholera toxin-induced ADP-ribosylation, is similar to that involved by nonhydrolyzable GTP analogues in the absence of cholera toxin treatment. Cholera toxin, however, does not influence the extent of GppNHp activation as is, indeed, expected. It should be emphasized that the GppNHp-induced activation retains its irreversible nature, whereas the enhanced GTP effect on the cholera toxin-treated membranes is reversible just as in untreated membranes. This is expected since cholera toxin treatment does not nullify the GTP hydrolytic step but just inhibits it.[75] The overall interrelationship between the adenylate cyclase and GTP is summarized in Figure 7.

F. The Dynamics of the Topographical Interrelationship Between Cyclase and the β-Receptor

Although it is quite well established that the three basic components of the adenylate cyclase system — the receptor, the G-protein (the GTP binding protein), and the catalytic unit — represent separate macromolecules, little is known about their organization within the membrane, their stoichiometry, and the mode of coupling between them. Certain theoretical arguments[81] favor the assumption that the stoichiometry is close to 1:1:1, but no direct proof is yet available as none of these components has been purified to homogeneity. However, more has been learned recently about the mechanism of coupling between these components in one experimental system, namely, the turkey erythrocyte β-adrenergic system. It is already established that the guanyl nucleotide regulatory unit is tightly attached to the catalytic unit and can only be separated from the latter upon solubilization in nonionic detergents and the biospecific absorption of the guanyl nucleotide protein onto a GTP-sepharose matrix. The β-receptor usually separates rather easily from the cyclase. The separation of the cyclase from the β-receptor upon membrane solubilization has been demonstrated in a number of cell types. In each case the solubilized cyclase could respond to GppNHp and to NaF, which indicates that the catalytic moiety and the GTP regulatory protein remain associated, even subsequent to solubilization. Thus the C_lG_m complex (C = enzyme, G = the GTP regulatory unit) is a stable structure with a yet unknown stoichiometry. Since the structural work on hormone-activated cyclases is progressing rather slowly, the approach taken to study the mode of coupling between receptors and adenylate cyclases has been mainly a kinetic one. We shall first consider the various theoretical models which have been formulated and then the experiments designed to explore the validity of these models.

G. Possible Modes of Receptor to Enzyme Coupling

In this section we shall consider the mode of coupling of β-adrenergic receptor to

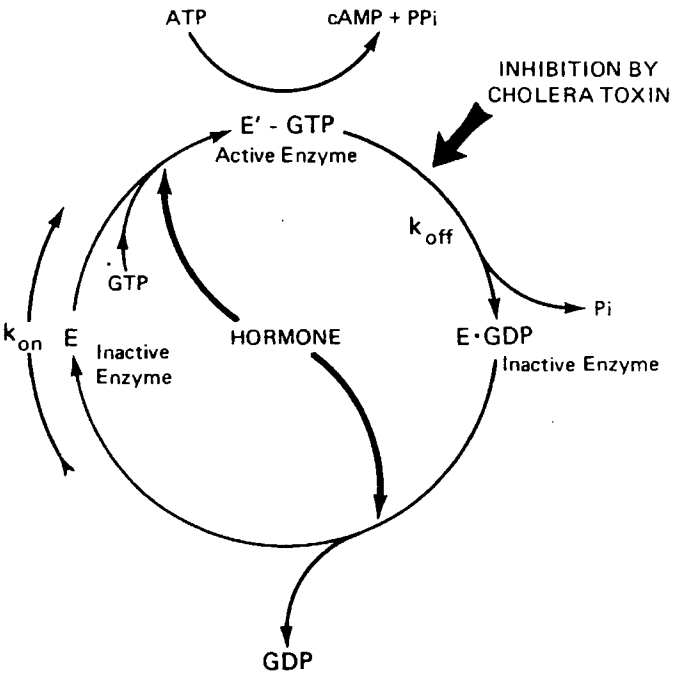


FIGURE 7. The role of GTP in adenylate cyclase activation. E'. GTP is the active species of the enzyme. k_{off} is the GTPase step whereas k_{on} is the rate constant characterizing the transition of the inactive enzyme E.GDP to the active state. The activation involves the removal of GDP from the regulatory site. This figure was drawn from the model developed by Levitzki[47] and Cassel and Selinger.[75]

adenylate cyclase. Whenever we refer to the "enzyme", we mean the whole complex between the catalytic moiety and the GTP regulatory protein. The theoretical considerations are usually applicable to other receptor cyclase systems and other receptor signal systems where the signal is not cyclase. Such a signal can be on transport via an ionophore coupled to the receptor. The experimental data discussed below will be confined to the β-receptor-dependent adenylate cyclase system, not only because it is the subject of this review, but also because detailed experiments aimed at the delineation of receptor to signal coupling have been thus far performed only on that system. In principle, four possible modes of coupling can occur:[48]

1. The precoupled model where the enzyme unit and the receptor regulatory unit are permanently attached to one another, as in the regulatory enzyme aspartate transcarbamylase. In this case the process of enzyme activation can be described in the upper scheme of Figure 9. The model predicts noncooperative agonist and antagonist binding. This model also predicts that the time course of enzyme activation to its permanently active state ($k_{off} = 0$), in the presence of hormone and GppNHp, is first order.[48]
2. The dissociation model assumes that the enzyme and the receptor are permanently attached to each other in the absence of agonist and that subsequent to hormone binding, the two units separate concomitantly with enzyme activation. This model predicts negatively cooperative ligand binding as well as nonfirst-order kinetics of enzyme activation when exposed to agonist in the presence of GppNHp.[48]

3. The floating receptor model assumes that the hormone, the receptor, and the enzyme are in equilibrium and, thus, the fraction of enzyme attached to the receptor can vary with the amount of agonist in the system. This model, like model II, predicts negatively cooperative hormone binding and complex kinetics of enzyme activation.[48]
4. The collision coupling model is described in the bottom section of Figure 8. This model predicts first-order kinetics of enzyme activation by hormone and GppNHp, and noncooperative ligand binding, as model 1.[48]

In one system, the turkey erythrocyte β-receptor-dependent cyclase, experiments were performed to explore the nature of the coupling between the receptor and the enzyme. Binding experiments of β-antagonists and β-agonists reveal that the mode of binding is *always* noncooperative in the turkey erythrocyte system.[33,34,38,48,82] Furthermore, the kinetics of enzyme activation in the presence of GppNHp is first order.[48,73] These two observations, even taken separately, constitute a firm basis to reject model 2 and model 3 for the turkey erythrocyte system. Both the binding experiments of β-ligands and the kinetic experiments can be accounted for equally well by either model 1 and its diametrically opposed model 4. A closer look[48] at these two models reveals that model 1 predicts that in the presence of GppNHp ($k_{off} = 0$), the rate constant of enzyme activation (k_{on}) is *independent* of receptor concentration and that the maximal number of activatable catalytic units is *directly proportional* to the concentration of receptor. In contrast, the collision coupling model (model 4) predicts the opposite: the rate constant of enzyme activation is *directly proportional* to the concentration of intact receptor, whereas the maximal number of catalytic units which can be activated is *independent* of receptor concentration. Using an irreversible β-adrenergic blocker[48] (see Figure 6), it was possible to demonstrate that the turkey erythrocyte β-adrenergic receptors *are not* permanently attached to the cyclase, but activate the enzyme by the collision coupling mechanism. Using an identical approach to investigate the mode of coupling between the adenosine receptor and the cyclase in the turkey erythrocyte system, the opposite situation was found:[83] the progressive inactivation of the adenosine receptor by an irreversible blocker results in a proportional decrease in the maximal number of activatable catalytic units in the presence of GppNHp, but *no change* in the rate constant characterizing the activation process (k_{on}) is effected. The fact that the adenosine receptor and the β-receptor are coupled differently to the adenylate cyclase was in fact predicted on kinetic grounds (see Reference 84 and references therein).

The conclusion that the adenosine receptor is permanently attached to the cyclase, whereas the β-receptor is not, is corroborated by an entirely different experimental approach. The progressive increase in membrane fluidity by the insertion of *cis*-vaccenic acid causes a dramatic increase in the rate constant (k_{on}) of cyclase activation by the β-agonist bound β-receptor,[49] but no change in the rate constant characterizing the activation of cyclase by adenosine.[83,84] These findings corroborate the assertion that the process of adenylate cyclase activation by β-receptors is bimolecular whereas the activation by adenosine is monomolecular. Another interesting aspect is that in the turkey erythrocyte system it was also shown that the two receptors activate a common pool of adenylate cyclase molecules through a common GTP regulatory unit.[85,86]

H. The Effect of GTP on β-Receptors

A number of reports have shown that besides the role of GTP in the activation of adenylate cyclase, guanyl nucleotides also affect the interaction of the receptor with the agonists. Thus, GTP and its analogues were found to reduce the affinity of glucagon to its receptors.[87] The GTP-induced reduction in the affinity of the glucagon re-

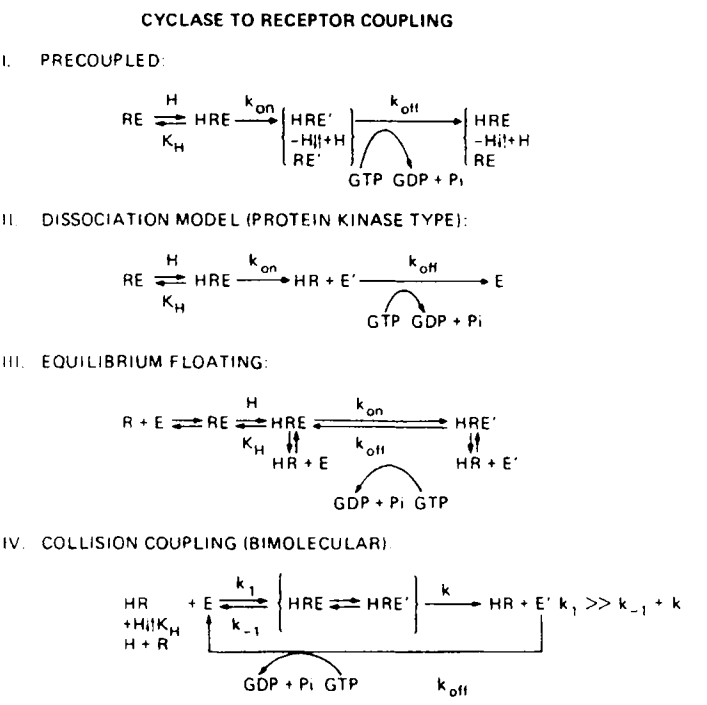


FIGURE 8. Possible modes of coupling between hormone receptors and adenylate cyclase.

ceptor toward the hormone is retained subsequent to its solubilization with Triton® X-100.[88] Similarly, GTP was shown in two cell types to reduce the affinity of β-adrenergic receptors toward β-agonists, but not toward β-blockers.[89] These effects of guanyl nucleotides are usually attributed to a receptor-linked nucleotide site which is different from the nucleotide site coupled to cyclase. The evidence for the existence of such a site, however, is so far indirect and further experimentation is necessary in order to confirm this assertion. On theoretical grounds, however, one can account for the effects on the GTP-receptor without assuming a new GTP site.[109]

I. The Role of the Lipid Matrix

Numerous studies reveal that the lipid matrix plays a role in the coupling between hormone receptors and adenylate cyclase. The treatment of membranes with phospholipases is known to damage the coupling between the hormone receptors and the cyclases. These observations may be taken as an indication that specific phospholipids are essential for the interaction between the hormone receptor and the cyclase and/or the G-protein. In the absence of more direct studies on this question, one cannot at the present time identify specific lipids essential for the function of adenylate cyclase. Similarly, the introduction of the membrane perturbing agent filipin[91] uncouples the β-adrenergic receptors from the cyclase and eliminates the ability of β-agonists to stimulate the cyclase, without affecting the binding of β-adrenergic ligands or the ability of NaF to activate the catalytic moiety of the cyclase. On the other hand, certain membrane fluidizing agents such as unsaturated fatty acids[49,92] or certain phospholipids[111] induce an increase in the efficiency of coupling between the β-adrenergic receptor and the cyclase in the turkey erythrocyte system. Similar findings were recently observed in cultured Chang liver cells.[93] Fluidization of cell membrane may also lead to the reversible cryptization[94] of receptors at higher temperatures, with a concomitant de-

crease in hormone-dependent adenylate cyclase activity.[94] This effect is separate from the effect of fluidization on the efficiency of coupling and can be dissected from it.

J. Future Directions

The research in the field of receptor to cyclase coupling is still in its infancy. Many key features of the adenylate cyclase regulatory system remain unknown. Let us list a few:

1. The structure of receptor-dependent cyclase regulatory complex is not as yet known. No data are available at present as to the stoichiometry among the three recognized macromolecules — the receptor, the GTP regulatory unit (the "transducer"), and the cyclase catalytic moiety. A further advance in this direction will be possible once these macromolecules will be purified and, subsequently, be reconstituted to a functional hormone-responsive cyclase under controlled experimental conditions. Progress in the field of structure of receptors is essential to further our understanding of the interactions between adenylate cyclase and its receptor. In addition, the fact that more than one kind of receptor may couple to a single pool of adenylate cyclase indicates that different receptors may possess a *common structural domain* with the enzyme (Figure 9). This situation might be taken as an indication that hormone receptors are built like antibodies and antigen receptors; namely, the receptor consists of a *variable* domain which represents the hormone binding site and a *constant* domain which represents the region of the receptor molecule that interacts with the GTP regulatory unit and/or the catalytic cyclase unit.
2. It is not clear whether all the components of the adenylate cyclase have already been discovered. For example, it is yet to be established whether the GTP regulatory site linked to the cyclase is identical with the site responsible for the hormone-dependent GTPase. Purification of the adenylate cyclase components and reconstitution experiments should yield some information on this point. Similarly, it is not yet clear whether the GTP-induced decrease in the affinity of certain hormone receptors coupled to cyclase toward the agonists is due to the interaction of the known GTP regulatory unit with the receptor, reflects the interaction of the receptor with still another GTP regulatory site.
3. How the process of hormone-induced desensitization (see also below) is linked to the coupling between the hormone receptor and the cyclase catalytic moiety is still an open question.
4. The role of specific membrane lipids as essential cofactors for the receptor to cyclase coupling must still be explored.
5. Whether the *fluidity* of the membrane matrix is a decisive factor in the mechanism of receptor to enzyme coupling in systems other than the few explored is not known as yet.
6. Connected to the last point is the question as to which of the hormone receptors known to activate adenylate cyclase are permanently associated to the cyclase and which are separate entities and therefore activate the enzyme during the transient encounter between the two. In the latter case, the receptors will not be found in the same domains as the enzyme as was indeed recently found in one system.[64] For the study of the relative distribution of hormone receptors *vis à vis* the cyclase, one will have to develop specific ultrastructural probes for the receptors as well as for the cyclase or the GTP binding protein.
7. The possibility that adenylate cyclase and the GTP regulatory unit, which face the inside of the cell, are anchored to cytoskeletal elements or are freely mobile

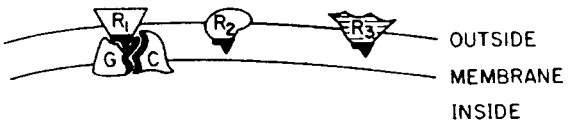


FIGURE 9. Adenylate cyclase related receptors may possess a common structural domain. The shaded area is the hypothetical domain which interacts with the catalytic moiety and/or the GTP regulatory unit. This so-called "constant" domain may have been conserved during evolution. Future structural work will of course reveal whether such a model is feasible.

in the lipid bilayer, must still be explored. Data on the latter point are relevant to the question whether receptor to enzyme coupling is also influenced by cytoskeletal elements.

X. SELF REGULATION OF β-ADRENERGIC RECEPTORS

A. Desensitization

The incubation of cells possessing β-adrenergic receptors leads to the decrease in receptor response. This feature of receptor behavior is common to many receptors and is known as receptor desensitization or subsensitivity. Lefkowitz and co-workers[95] demonstrated in intact frog erythrocytes that receptor desensitization is expressed in decreased β-agonist-dependent adenylate cyclase and/or reduction of the total number of β-receptors on the surface of the cell. Similar observations were made in a large variety of cell types. In most studies on a variety of cells it was noted that the degree of adenylate cyclase desensitization usually exceeds the degree of loss of β-receptors. A more detailed kinetic analysis on the mechanism of β-receptor desensitization of human astrocytoma cells (see Reference 96 and references therein) suggests that the phenomenon of densensitization involves two consecutive steps: (1) a rapid uncoupling of the β-receptors from the cyclase; namely, the loss of hormonal situation of adenylate cyclase, followed by (2) loss of β-adrenergic receptors from the cell surface. The initial event of the loss of receptor to enzyme coupling is reversible, whereas the loss of β-receptors from the surface of the cells is not. The disappearance of receptors from the frog erythrocyte surface was reported to occur together with the increase of β-receptors in the cytosol,[97] suggesting internalization as was found for polypeptide hormones such as insulin. The reappearance of β-receptors on the cell surface requires the synthesis of new receptors. It is not yet clear whether the internalized receptors are disintegrated or recycled. One can summarize the two-stage process of receptor desensitization by the scheme:

$$R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R' \longrightarrow R''$$

where R is the free unbound receptor, R′ is the agonist bound receptor in the uncoupled state, and R″ is the internalized receptor which is n longer accessible to ligands. The establishment of the steady state R⇌R′ seems to be rather fast, whereas the irreversible process of R′ to R″ conversion is slow and usually spans over a few hours. The R′ to R ratio depends on hormone concentration and increases with the efficacy of the agonist. The appearance of cytosolic receptor capable of binding ^{3}H-alprenolol[97] subsequent to the exposure of the cells to β-agonist indicates that the binding properties of

the receptor are retained. This behavior may be nontypical since the frog erythrocytes may lack degradative mechanisms which are normally operating in most if not all other cell types. Interestingly, turkey erythrocytes were found to lack the phenomenon of receptor desensitization altogether.[38] In certain cases it was shown that protein synthesis is required for β-receptor desensitization,[98] but its involvement in other cases was not recognized. It may be that a certain protein component is required for the process of long-term desensitization. The reason that cells differ in their dependence of long-term desensitization on protein synthesis may be that the yet unknown protein factor has different lifetimes in different cells.

For other hormones and certain other proteins (see Reference 99 and references therein), it was shown that the receptor internalization involves the collection of the receptor ligand complexes in "coated pits" where the latter pinch off to form endocytotic vesicles. It is not clear yet, however, whether the coated pit mechanism is involved in desensitization. It is also not yet clear whether receptor desensitization to catecholamines follows a coated pit type of internalization. Interestingly enough, the ultrastructural localization of β-receptors on muscle cells shows a high concentration of ligand receptor complexes near microvilli,[51] where most coated pits are usually found.[52] The circadian cycle in the responsiveness of the pineal organ to catecholamines was found to be responsible for the circadian rhythm of melatonin biosynthesis by the gland. The circadian nature of the increase-decrease in the responsiveness of the gland to catecholamines results in the circadian change in the responsiveness of the adenylate cyclase to β-adrenergic stimulation and the corresponding oscillatory change in the number of β-adrenergic receptors.[100,101]

B. β-Receptor Supersensitivity

Supersensitization of β-adrenergic receptors at target cells occurs in nerve terminals as a compensatory process following the reduction or the complete inhibition of catecholamine release from the nerve ending. Supersensitization of the β-adrenergic receptors can occur in a target organ which responds to catecholamines arriving as a hormone or a result of a decrease in the input of catecholamines. Supersensitization is manifested both in increased cAMP synthesis and in increased level of β-receptors. It was found that subsequent to adrenalectomy of the rat the responsiveness of rat liver adenylate cyclase to catecholamines was enhanced three- to fivefold.[102] This increased responsiveness is accompanied by a three- to fivefold increase in the number of β-adrenergic receptors, as revealed by direct binding studies using ^{125}I-hydroxybenzylpindolol. These changes are reversed by the administration of cortisone. It was suggested[102] that this increase in β-receptors and adenylate cyclase activity are a compensatory response to the impairment in gluconeogenesis and glycolysis, which occur subsequent to adrenalectomy. Changes in catecholamine responsiveness in the mammalian brain occur as a result of intraventricular injection of 6-hydroxydopamine which causes a destruction of catecholaminergic nerve terminals and therefore a reduction or a complete cessation of catecholamine output. Using ^{125}I-hydroxybenzylpindolol, one can detect an increase in the number of β-receptors as well as an increase in the β-receptor increase of the l-isoproterenol-dependent adenylate cyclase.[103] The pineal gland is another system where one can study the regulation f adrenergic function.[104] In this system, a period of increased adrenergic stimulation leads to a diminished response to subsequent stimulation, namely, subsensitivity. This period is followed by a period of reduced stimulation which, in turn, leads to an increased β-adrenergic response, namely, supersensitivity. Glands taken from animals after 12 or 24 hr of exposure to light are supersensitive relative to glands taken from animals at the end of their daily exposure to 12 hr of darkness.[105] Data have accumulated to

indicate that the sensitivity of β-adrenergic stimulation is expressed in multiple sites: (1) the number of β-receptor sites, (2) the response of adenylate cyclase to β-agonists, (3) the levels of cAMP phosphodiesterase, and (4) the level of cAMP-dependent protein kinase. All these biochemical signals were shown to follow a circadian cycle of sub- and supersensitivity. As for the case of desensitization, the investigation of the time course of the different events may shed more light on the molecular parameters involved in the onset of a state of supersensitivity.

X. CONCLUSION

From the discussion of the coupling of adenylate cyclase to β-receptors, it is apparent that much is yet to be learned about the cyclase system. Many of the features described here for β-receptor-dependent cyclase are common to other receptor-dependent cyclases. It is also clear that the mechanism of action and control of receptor-dependent cyclases is far from being fully understood. It remains for future studies to establish a number of key features of the receptor-dependent adenylate cyclase. It is almost impossible to conceive progress in this field without the purification of the components involved in the action and control of adenylate cyclase and serious attempts to reconstitute them. Many receptors seem to regulate adenylate cyclase such that their occupancy results in inhibition of the enzyme. It seems that one can divide the cyclase-linked receptors to two families: (1) the cyclase activating receptors which include the β-receptors, and (2) the cyclase inhibitory receptors which include α_2 receptors, inhibitory adenosine receptor, and one class of morphine-enkephaline receptors. Furthermore, protein components other than receptors seem to participate in the control of the cAMP output by adenylate cyclase — to name a few, the GTP regulatory protein(s) and calmodulin. It seems, therefore, that adenylate cyclase is the core of a multicomponent system of which the activating receptor such as the β-receptor is only one segment. It is therefore safe to state that most of the discoveries in this field are yet to be made and an exciting time is awaiting the investigators who will possess the perseverance to stick with the problem.

REFERENCES

1. **Furchgott, R. F.**, The classification of adrenoreceptors. An evaluation from the standpoint of receptor theory, in *Handbook of Experimental Pharmacology: Catecholamines*, Vol. 33, Springer-Verlag, Berlin, 1972, 283.
2. **Langer, S. Z.**, Presynaptic receptors and their role in the regulation of transmitter release, *J. Pharmacol.*, 60, 481, 1977.
3. **Kebabian, J. W.**, Multiple receptors for dopamine, *Nature (London)*, 277, 93, 1979.
4. **Sutherland, E. W., Pye, I., and Butcher, R. W.**, The action of epinephrine and the role of the adenyl cyclase system in hormone action, *Recent Prog. Horm. Res.*, 21, 623, 1965.
5. **Levitzki, A.**, The mode of coupling between adenylate cyclase to hormone receptors and its modulation by GTP, *Biochem. Pharmacol.*, 27, 2081, 1979.
6. **Steer, M. L. and Levitzki, A.**, The interaction of catecholamines, Ca^{2+} and adenylate cyclase in the intact turkey erythrocytes, *Arch. Biochem. Biophys.*, 167, 371, 1975.
7. **Rassmussen, H., Lake, W., and Allen, J. E.**, The effect of catecholamines and prostaglandin upon human and rat erythrocytes, *Biochim. Biophys. Acta*, 411, 63, 1975.
8. **Steer, M. L. and Levitzki, A.**, The control of adenylate cyclase by calcium in turkey erythrocyte ghosts, *J. Biol. Chem.*, 250, 2080, 1975.
9. **Hanski, E., Sevilla, N., and Levitzki, A.**, The allosteric inhibition by calcium of soluble and partially purified adenylate cyclase from turkey erythrocytes, *Eur. J. Biochem.*, 76, 512, 1977.

10. **Schramm, M. and Seligner, Z.,** The functions of cyclic AMP and calcium as alternative second messengers in parotid gland and pancreas, *J. Cycl. Nucl. Res.*, 1, 181, 1975.
11. **Jacobs, K. H.,** Inhibition of platelet adenylate cyclase by α-adrenergic agonists, in *Molecular Biology and Pharmacology of Cyclic Nucleotides,* Falco, G. and Poletti, R., Eds., Elsevier, Amsterdam, 1978, 265.
12. **Sabol, S. L. and Nirenberg, M.,** Regulation of adenylate cyclase of neuroblastoma x glioma hybrid cells by α-adrenergic receptors, *J. Biol. Chem.*, 254, 1913, 1979.
13. **Steer, M. L. and Wood, A.,** Regulation of human platelet adenylate cyclase by epinephrine, prostaglandin E, guanine nucleotides, *J. Biol. Chem.*, 254, 10791, 1978.
14. **Hirata, F., Strittmatter, J., and Axelrod, J.,** β-Adrenergic receptor agonist increase phospholipid methylation, membrane fluidity and β-adrenergic receptor adenylate cyclase coupling, *Proc. Natl. Acad. Sci. U.S.A.*, 76, 368, 1979.
15. **Strittmatter, W. J., Gagnon, C., and Axelrod, J.,** Beta adrenergic stimulation of protein carboxymethylation and amylase secretion in rat parotid gland, *J. Pharmacol. Exp. Ther.*, 207, 419, 1979.
16. **Haylett, D. G. and Jenkinson, D. H.,** The receptors concerned in the actions of catecholamines on glucose release, membrane potential and ion movements in guinea pig liver, *J. Physiol. (London)*, 225, 751, 1972.
17. **Girardier, L., Seydoux, J., and Clausen, T.,** Membrane potential of brown adipose tissue: A suggested mechanism for the regulation of thermogenesis, *J. Gen. Physiol.*, 52, 925, 1968.
18. **O'Dea, R. F. and Zatz, M.,** Catecholamine stimulated cGMP accumulation in the rat pineal, apparent presynaptic site of action, *Proc. Natl. Acad. Sci. U.S.A.*, 73, 3398, 1976.
19. **Daly, J.,** Role of cyclic nucleotides in the nervous system, in *Handbook of Psychopharmacology,* Vol. 5, Iversen, L. L., Iversen, S. D., and Snyder, S. H., Eds., Plenum Press, New York, 1975, 47.
20. **Sharma, S. K., Klee, W. A., and Nirenberg, M.,** Opiate-dependent inhibition of adenylate cyclase, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 3365, 1977.
21. **Blume, A. J. and Boone, G.,** Effective coupling of opiate receptors to adenylate cyclase: requirement for nucleoside triphosphates and monovalent cations, *Fed. Proc.*, 38, 628, 1979.
22. **Londos, C., Cooper, D. M. F., Schlegel, W., and Rodbell, M.,** Adenosine analogs inhibit adipocyte adenylate cyclase by a GTP-dependent process, *Proc. Natl. Acad. Sci. U.S.A.*, 75, 5362, 1978.
23. **Oron, Y., Lowe, M., and Selinger, Z.,** Incorporation of inorganic [^{32}P] phosphate into rat parotid phosphatidylinositol, *Mol. Pharmacol.*, 11, 79, 1975.
24. **Batzri, S., Selinger, Z., Schramm, M., and Robinovitch, M. R.,** Potassium release mediated by the epinephrine α-receptor in the rat parotid slices, *J. Biol. Chem.*, 248, 361, 1975.
25. **Kunos, G. and Nickersen, M.,** Temperature induced interconversion of α and β adrenoreceptors in the frog heart, *J. Physiol. (London)*, 256, 23, 1973.
26. **Caron, M. G. and Lefkowitz, R. J.,** Temperature immutability of adenyl cyclase coupled β-adrenergic receptors, *Nature*, 249, 258, 1947.
27. **Prasad, K. N. and Gilmer, K. N.,** Demonstration of dopamine sensitive adenylate cyclase in malignant neuroblastoma cells and change in sensitivity of adenylate cyclase to catecholamines in "differentiated" cells, *Proc. Natl. Acad. Sci. U.S.A.*, 71, 2525, 1974.
28. **Horn, A. S., Curello, A. C., and Miller, R. J.,** Dopamine in the mesolimlic system of the rat brain: endogenous effects and the effects of drugs on the uptake mechanism and stimulation of adenylate cyclase activity, *J. Neurochem.*, 1974.
29. **Burt, D. R., Cresse, I., and Synder, S. H.,** Binding interactions of lysergic acid diethylamide and related agents with dopamine receptors in the brain, *Mol. Pharmacol.*, 12, 631, 1976.
30. **Williams, L. T. and Lefkowitz, R. J.,** Alpha-adrenergic receptor identification by [^{3}H]dihydroergocryptine binding, *Science*, 192, 739, 1976.
31. **Levitzki, A., Atlas, D., and Steer, M. L.,** The binding characteristics and number of β-adrenergic receptors on the turkey erythrocyte, *Proc. Natl. Acad. Sci. U.S.A.*, 71, 2773, 1975.
32. **Cuatrecasas, P., Tell, G. P. E., Sica, V., Parikh, I., and Chang, K. J.,** Noradrenaline binding and the search for catecholamine receptors, *Nature (London)*, 247, 92, 1974.
33. **Atlas, D., Steer, M. L., and Levitzki, A.,** Stereospecific binding of propranolol and catecholamines to the β-adrenergic receptor, *Proc. Natl. Acad. Sci. U.S.A.*, 71, 4246, 1974.
34. **Aurbach, G. D., Fedak, S. A., Woodward, C. J., Palmer, J. S., Hauser, D., and Troxler, F.,** β-Adrenergic receptor: stereospecific interaction of iodinated β-blocking agent with high affinity site, *Science*, 186, 1223, 1974.
35. **Lefkowitz, R. J., Mukherjee, C., Coverstone, M., and Caron, M. G.,** Stereospecific [^{3}H] (−) alprenolol binding sites, β-adrenergic receptors and adenylate cyclase, *Biochem. Biophys. Res. Commun.*, 60, 703, 1974.
36. **Levitzki, A.,** Catecholamine receptors, *Rev. Physiol. Biochem. Pharmacol.*, 82, 1, 1978.

37. **Vauquelin, G., Geynet, P., Hanoune, J., and Strossberg, D.,** Isolation of adenylate cyclase free, β-adrenergic receptor from turkey erythrocyte membrane, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 3710, 1977.
38. **Hanski, E. and Levitzki, A.,** The absence of desensitization in the β-adrenergic receptors of turkey reticulocytes and erythrocytes and its possible origin, *Life Sci.*, 22, 53, 1978.
39. **Lefkowitz, R. J. and Williams, L. T.,** Catecholamine binding to the beta-adrenergic receptor, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 515, 1977.
40. **Atlas, D. and Levitzki, A.,** Probing of β-adrenergic receptors by novel fluorescent β-adrenergic blockers, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 5290, 1977.
41. **Melamed, E., Lahav, M., and Atlas, D.,** Direct localization of β-adrenoreceptor sites in rat cerebellum by a new fluorescent analogue of propranolol, *Nature (London)*, 261, 420, 1976.
42. **Atlas, D., Terchberg, V. I., and Changeux, J. P.,** Direct evidence for beta adrenoreceptors on the Reokinje cells of mouse cerebellum, *Brain Res.*, 28, 532, 1976.
43. **Atlas, D.,** Fluorescence mapping of β-adrenergic receptors, in *Perspectives in Nephrology and Hypertension*, Schmitt, M. and Mayer, P., Eds., John Wiley & Sons, New York, 1979.
44. **Falck, B., Hillarp, P. N. A., Thieme, G., and Torp, A.,** Fluorescence of catecholamines and related compounds condensed with formaldehyde, *J. Histochem. Cytochem.*, 10, 348, 1962.
45. **Lindvall, O. and Bjorklund, A.,** The organization of the ascending catecholamine neuron systems in the rat brain as revealed by glyoxylic acid fluorescent method, *Acta Physiol. Scand.*, 92 (Suppl.), 1, 1974.
46. **Orly, J. and Schramm, M.,** Coupling of catecholamine receptor from one cell with adenylate cyclase from another cell by cell fusion, *Proc. Natl. Acad. Sci. U.S.A.*, 73, 4410, 1976.
47. **Eimerl, S., Neufeld, G., Korner, M., and Schramm, M.,** Functional implantation of a solubilized β-adrenergic receptor in the membrane of a cell, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 760, 1980.
48. **Tolkovsky, A. M. and Levitzki, A.,** The mode of coupling between β-adrenergic receptor and adenylate cyclase in turkey erythrocytes, *Biochemistry*, 2, 3795, 1978.
49. **Hanski, E., Rimon, G., and Levitzki, A.,** Adenylate cyclase activation by the β-adrenergic receptor as a diffusion controlled process, *Biochemistry*, 18, 846, 1979.
50. **Schlessinger, Y., Schechter, Y., Willingham, M. C., and Pastan, I.,** Direct visualization of the binding, aggregation and internalization of insulin and epidermal growth factor in fibroblastic cells, *Proc. Natl. Acad. Sci. U.S.A.*, 75, 2659, 1978.
51. **Atlas, D., Yaffe, D., and Skutelstky, E.,** The ultrastructural probing of β-adrenoreceptors on cell surfaces, *FEBS Lett.*, 95, 173, 1973.
52. **Willingham, M. C., Maxfield, F. R., and Pastan, I.,** α_2-Macroglobulin binding to the plasma membrane of cultured fibroblasts: diffuse binding followed by clustering in coated regions, *J. Cell Biol.*, in press.
53. **Atlas, D. and Llvitzki, A.,** An irreversible blocker for the β-adrenergic receptor, *Biochem. Biophys. Res. Commun.*, 69, 397, 1976.
54. **Atlas, D., Steer, M. L., and Levitzki, A.,** An affinity label for the β-adrenergic receptor in turkey erythrocyte, *Proc. Natl. Acad. Sci. U.S.A.*, 73, 1921, 1976.
55. **Atlas, D. and Levitzki, A.,** The tentative identification of the β-adrenoreceptor subunits, *Nature (London)*, 272, 5651, 1978.
56. **Hagar, T., Hagar, K., and Gilman, A. G.,** Hydrodynamic properties of the β-adrenergic receptor and adenylate cyclase from wild type and variant 543 lymphoma cells, *J. Biol. Chem.*, 252, 5776, 1977.
57. **Limbrid, L. E. and Lefkowitz, R. J.,** Resolution of β-adrenergic receptor binding and adenylate cyclase activity of gel exclusion chromatography, *J. Biol. Chem.*, 252, 779, 1977.
58. **Darfler, F. J. and Marinetti, G. V.,** Synthesis of a photoaffinity label for the beta adrenergic receptor, *Biochem. Biophys. Res. Commun.*, 79, 1, 1977.
59. **Takaganogi, I., Yoshioka, M., Takagi, K., and Tamura, Z.,** Photoaffinity labeling of the β-adrenergic receptors and receptor reserves for isoprenaline, *Eur. J. Pharmacol.*, 35, 121, 1976.
60. **Wrenn, S. M. and Haber, E.,** Production and characterization of an antibody which specifically inhibits the function of the cardiac β-adrenergic receptor, *Fed. Proc.*, 35 (Abstr. 1410), 1634, 1976, Ab. 1410.
61. **Caron, M. G., Srimvasan, Y., Pitha, J., and Lefkowitz, R. J.,** Affinity chromatography of the β-adrenergic receptor, *J. Biol. Chem.*, 254, 2923, 1979.
62. **Robison, G. A., Butcher, R. W., and Sutherland, E. W.,** Adenyl cyclase as an adrenergic receptor, *Ann. N.Y. Acad. Sci.*, 139, 703, 1967.
63. **Levitzki, A.,** Catecholamine receptors: group report, in *Hormone and Antihormone Action at the Target Cell*, Clark, J. M., Klee, W., Levitzki, A., and Wolf, J., Eds., Dahlem Conference, Berlin, 1976, 73.

64. **Sahyoun, N., Hollenberg, M. D., Bennet, V., and Cuatrecasas, P.,** Topographic separation of adenylate cyclase and hormone receptors in the plasma membrane of toad erythrocyte ghosts, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 2860, 1977.
65. **Howlett, A. C., Sternweis, P. C., Mauk, B. A., Van Arsdale, P. M., and Gilman, A. G.,** Reconstitution of catecholamine sensitive adenylate cyclase, *J. Biol. Chem.*, 254, 2287, 1979.
66. **Rodbell, M., Lin, M. C., Salomon, Y., Londos, C., Marwood, J. P., Martin, B. P., Rendell, M., and Berman, M.,** The role of adenine and guanine nucleotides in the activity and response of adenylate cyclase systems to hormones: evidence for multi-site transition state, *Acta Endocrinol. (Copenhagen) Suppl.*, 191, 11, 1974.
67. **Levitzki, A.,** The role of GTP in the activation of adenylate cyclase, *Biochem. Biophys. Res. Commun.*, 74, 1154, 1977.
68. **Levitzki, A. and Helmreich, E. J. M.,** Hormone-receptor adenylate cyclase interactions, *FEBS Lett.*, 101, 213, 1979.
69. **Arad, H. and Levitzki, A.,** The mechanism of partial agonism in the β-receptor dependent adenylate cyclase of turkey erythrocytes, *Mol. Pharmacol.*, 16, 743, 1979.
70. **Pfeuffer, T. and Helmreich, E. J. M.,** Activation of pigeon erythrocyte membrane adenylate cyclase by guanyl nucleotide analogues and separation of a nucleotide binding protein, *J. Biol. Chem.*, 250, 867, 1975.
71. **Cassel, D. and Selinger Z.,** Catecholamine-stimulated GTPase activity in turkey erythrocyte membranes, *Biochim. Biophys. Acta*, 452, 538, 1976.
72. **Blume, A. and Foster, C. J.,** Neuroblastoma adenylate cyclase, role of 2-chloradenosine, prostaglandin E_1, and guanine nucleotides in regulation of activity, *J. Biol. Chem.*, 251, 3399, 1976.
73. **Sevilla, N., Steer, M. L., and Levitzki A.,** The synergistic activation of adenylate cyclase by guanylyl imidodiphosphate and epinephrine, *Biochemistry*, 15, 3493, 1976.
74. **Cassel, D., Eckstein, F., Lowe, M., and Selinger, Z.,** Determinations of the turn-off reaction for the hormone activated adenyylate cyclase, *J. Biol. Chem.*, 254, 9835, 1979.
75. **Cassel, D. and Selinger, Z.,** Mechanism of adenylate cyclase activation by cholera toxin: inhibition of GTP hydrolysis at the regulatory site, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 3307, 1977.
76. **Pfeuffer, T.,** GTP-binding proteins in membranes and the control of adenylate cyclase activity, *J. Biol. Chem.*, 252, 7224, 1977.
77. **Pfeuffer, T.,** Guanine nucleotide-controlled interactions between components of adenylate cyclase, *FEBS Lett.*, 101, 85, 1979.
78. **Moss, J. and Vaughan, M.,** Mechanism of action of cholarogen: evidence for ADP-ribosyltransferase activity with arginine as an acceptor, *J. Biol. Chem.*, 252, 2455, 1977.
79. **Gill, D. M. and Meren, R.,** ADP-ribosylation of membrane proteins catalyzed by cholera toxin: basis of the activation of adenylate cyclase, *Proc. Natl. Acad. Sci. U.S.A.*, 75, 3050, 1978.
80. **Cassel, D. and Pfeuffer, T.,** Mechanism of cholera toxin action: covalent modification of the guanyl nucleotide binding protein of adenylate cyclase, *Proc. Natl. Acad. Sci. U.S.A.*, 75, 2669, 1978.
81. **Levitzki, A., Sevilla, N., Atlas, D., and Steer, M. L.,** Ligand specificity and characteristics of the β-adrenergic receptor in turkey erythrocyte plasma membrane, *J. Mol. Biol.*, 37, 35, 1975.
82. **Brown, E. M., Fedak, S. A., Woodard, C. J., Aurbach, G. D., and Rodbard, D.,** β-Adrenergic receptor interactions. Direct comparison of receptor interaction and biological activity, *J. Biol. Chem.*, 251, 1239, 1976.
83. **Braun, S. and Levitzki, A.,** The simultaneous coupling of adenylate cyclase to the β-adrenergic receptor and the adenosine receptor, *Biochemistry*, 17, 3811, 1979.
84. **Tolkovsky, A. M. and Levitsky, A.,** Coupling of a single adenylate cyclase to two receptors: adenosine and catecholamine, *Biochemistry*, 17, 3811, 1978.
85. **Rimon, G. Hanski, E., Braun, S., and Levitzki, A.,** Mode of coupling between hormone receptors elucidated by modulation of membrane fluidity, *Nature (London)*, 276, 394, 1978.
86. **Sevilla, N., Tolkovsky, A. M., and Levitzki, A.,** The activation of adenylate cyclase by l-epinephrine and guanylylimido-phosphate and its reversal by l-epinephrine and GTP, *FEBS Lett.*, 81, 339, 1977.
87. **Arad, H., Rimond, G., and Levitzki, A.,** The reversal of the GPPNHP permanently active state of adenylate cyclase by GTP and hormone by the "collision coupling" mechanism, *J. Biol. Chem.*, in press.
88. **Rodbell, M., Krans, H. M. J., Phol, S. L., and Birnbaumer, L.,** The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. III. Binding of glucagon — method of assay and specificity, *J. Biol. Chem.*, 246, 1861, 1971.
89. **Welton, A. F., Lad, P. M., Newby, A. C., Yamamura, M., Nicosia, S., and Rodbell, M.,** Solubilization and separation of the glucagon receptor and adenylate cyclase in guanine nucleotide-sensitive states, *J. Biol. Chem.*, 252, 5947, 1977.

90. **Maguire, M. E., Van Arsdale, P. M., and Gilman, A. G.,** An agonist specific effect of guanine nucleotides on binding to the beta-adrenergic receptor, *Mol. Pharmacol.*, 12, 335, 1976.
91. **Puchwein, G., Pfeuffer, T., and Helmreich, E. J. M.,** Uncoupling of catecholamine activation of pigeon erythrocyte membrane adenylate cyclase by filipin, *J. Biol. Chem.*, 249, 3232, 1974.
92. **Orly, J. and Schramm, M.,** Fatty acids as modulators of membrane functions: catecholamine activated adenylate cyclase of the turkey erythrocyte, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 3433, 1975.
93. **Bakardjieva, A., Galla, M. J., and Helmreich, E. J. M.,** Modulation of the β-receptor adenylate cyclase interactions in cultured Chang liver cells by membrane fluidization, *Biochemistry*, 18, 3016, 1979.
94. **Rimon, G., Hanski, E., and Levitzki, A.,** The temperature dependencies of the coupling between adenylate cyclase and the β-receptors and adenosine receptors, *Biochemistry*, 19, 4451, 1980.
95. **Lefkowitz, R. J., Mulliken, D., and Caron, M. G.,** Regulation of β-adrenergic receptors by guanyl-5′-yl imidodiphosphate and other purine nucleotides, *J. Biol. Chem.*, 251, 4686, 1976.
96. **Harden, T. K., Su, Y. F., and Perkins, J. P.,** Catecholamine induced desensitization involves an uncoupling of β-adrenergic receptors and adenylate cyclase, *J. Cycl. Nucl. Res.*, 5, 99, 1979.
97. **Chang, D. W. and Costa, E.,** Evidence for internalization of the recognition site of β-adrenergic receptor during receptor subsensitivity induced by (−) isoproterenol, *Proc. Natl. Acad. Sci. U.S.A.*, 76, 3024, 1979.
98. **De Vellis, J. and Brooker, G.,** Reversal of catecholamine refractoriness by inhibitors of RNA and protein synthesis, *Science*, 186, 1221, 1974.
99. **Goldstein, J. L., Anderson, R. G. W., and Brown, M. S.,** Coated pits, coated vesicles and receptor-mediated endocytosis, *Nature (London)*, 279, 679.
100. **Kebabian, J. W., Zatz, M., Romero, J. A., and Axelrod, J.,** Rapid changes in rat pineal β-adrenergic receptor. Alterations in 1-[^{3}H]-alprenalol binding and adenylate cyclase, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 3735, 1975.
101. **Romero, J. A., Zatz, M., Kebabian, J. W., and Axelrod, J.,** Circadian cycles in binding of ^{3}H-alprenolol to β-adrenergic receptor sites in rat pineal, *Nature (London)*, 258, 435, 1975.
102. **Wolfe, B. B., Harden, T. K., and Molinoff, P. B.,** β-Adrenergic receptors in rat liver: effects of adrenalectomy, *Proc. Natl. Acad. Sci. U.S.A.*, 73, 1343, 1976.
103. **Spring, J. R., Harden, T. K., Wolfe, B. B., and Molinoff, P. B.,** Adrenergic involvement in 6-hydroxydopamine induced supersensitivity in rat cerebral cortex, *Science*, 194, 624, 1976.
104. **Axelrod, J.,** The pineal gland: a neurochemical transducer, *Science*, 184, 1341, 1974.
105. **Romero, J. A. and Axelrod, J.,** Pineal β-adrenergic receptor: diurnal variation in sensitivity, *Science*, 184, 1091, 1974.
106. **Axelrod, J.,** personal communication.
107. **Helmreich, E. J. M.,** personal communication.
108. **Atlas, D.,** unpublished experiments.
109. **Schramm, M.,** personal communication.
110. **Cassel, D. and Pfeuffer, T.,** unpublished data.
111. **Levitzki, A.,** unpublished data.
112. **Hanski, E.,** unpublished data.