

Adrenoceptors: Molecular Nature and Role in Atopic Diseases

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Since Szentivanyi proposed the idea that asthma and other atopic diseases are due to a β adrenergic defect there has been much interest in the role of the adrenergic receptors in allergy. The radioactive ligand binding techniques developed within the last few years have greatly increased our knowledge concerning the molecular nature of the adrenoceptors and the events following receptor stimulation. The adrenoceptors have shown to be very dynamic structures. Their number and affinity may be altered due to various physiological and pharmacological stimuli. Their role in the pathogenesis of atopic diseases has not been definitely settled, but there seem to be a true beta adrenergic hyporesponsiveness and alpha hyperresponsiveness in asthma. This article briefly describes the radioligand binding technique and summarizes our present knowledge of the nature of the alpha and beta adrenoceptors and their possible role in atopic diseases.

Key words: adrenergic receptors; atopy; asthma.

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Functional subclassification of the adrenoceptors

It has been known since the beginning of the century that the sympathomimetic amines have both excitatory and inhibitory effects in the organism. Early investigators proposed the existence of two fundamental types of mediator substances: an excitatory sympathin E and an inhibitory sympathin I (1). As no amine which fulfilled the requirements for either sympathin E or I could be found, the idea of two different receptor types emerged. On the basis of the relative potency of six adrenergic amines on various biological (mainly haemodynamic) parameters, Ahlquist (1) defined two different receptors: an α receptor which mediates most of the adrenergic amines' excitatory functions (vasoconstriction, pupil dilation, uterine contraction) and a few inhibitory (intestinal relaxation), and a β receptor which mediates most of the inhibitory functions (vasodilation, bronchodilation, uterine relaxation) and a few excitatory (cardiac inotropy and chronotropy).

An α receptor is defined as a receptor for which epinephrine is a more potent stimulator (agonist) than norepinephrine which again is more potent than isoproterenol. The potency of the amines was examined in intact animals or isolated organ preparations. In contrast, the β receptor is defined as a receptor for which isoproterenol is a more potent agonist than epinephrine which again is more potent than norepinephrine. The development of relatively selective α and β antagonists has further confirmed the pharmacological usefulness of the α and β receptor concept. Table 1 summarizes the major pharmacological characteristics of the α and the β receptor.

In 1967, Lands (55) showed that there is marked similarity between the relative potencies of sympathomimetic amines on lipolysis and cardiac stimulation on the one hand and on bronchodilation and vasodepression on the other. Furthermore, a potent lipolytic and cardiac stimulating amine is a poor bronchodilator and vasodepressor. Therefore, Lands sug-

Table 1

Pharmacological characteristics of α and β receptors		
Subject	α Receptors	β Receptors
Agonist potency	Epi > Nepi > Iso	Iso > Epi > Nepi
Physiological effects	Most excitatory	Most inhibitory
Specific antagonist	Phentolamine	Propanolol

Abbreviations: Epi: epinephrine; Nepi: norepinephrine; Iso: isoproterenol.
Specific examples of the physiological effects mediated by α and β receptors respectively are given in Tables 2 and 3.

Table 2.

Characteristics of β_1 and β_2 receptors		
Subject	β_1 Receptors	β_2 Receptors
Agonist potency	Iso > Epi = Nepi	Iso > Epi > Nepi
Specific agonist ^a	Dobutamine	Salbutamol
Specific antagonist ^a	Practolol	Butoxamine
Major effects	Increases cardiac contractility and rate Increases lipolysis	Bronchodilation Vasodilation Increases muscle glycogenolysis Increases muscle tremor Inhibits histamine release Inhibits antibody response Inhibits T-lymphocyte cytolytic activity Inhibits polymorph degranulation

^a Only one example of each drug type is given. The specificity is only relative and based on *in vivo* experiments. For the drug cited (except dobutamine) the specificity has been confirmed by *in vitro* experiments (66).

gested the existence of two different types of β receptors, called β_1 and β_2 receptors. The β receptors are defined by their relative affinities for epinephrine and norepinephrine (Table 2). Epinephrine and norepinephrine are equally potent stimulators of the beta₁ receptors which mainly mediate cardiac stimulation and lipolysis. In contrast, epinephrine is a much more potent β_2 stimulator than norepinephrine. The β_2 receptors mediate bronchodilation and vasode-

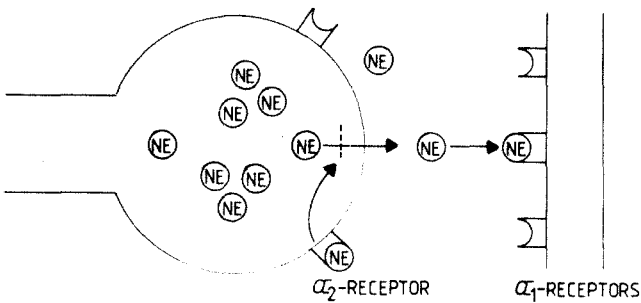


Fig. 1. α_1 and α_2 receptors. The α_1 receptors are located postsynaptically and mediate smooth muscle contraction. The α_2 receptors are often located presynaptically. Stimulation of a presynaptic α_2 receptor in the adrenergic system inhibits further norepinephrine (NE) release.

pression and inhibit the activity of several of the cell types in the immune system (Table 2), among other effects.

Recently, it has become clear that the α receptors also can be subclassified (see (12) for a review). So it has been suggested (12) that the α receptor should be subdivided into (at least) two groups, α_1 and α_2 receptors. α_1 Receptors are defined as α receptors mediating smooth muscle constriction (vasoconstriction). Methoxamine and phenylephrine are relatively selective α_1 receptor stimulators. The α_1 receptors seem to be located postsynaptically in every case (Fig. 1).

The α_2 receptors are the α receptors typically mediating the inhibition of neurotransmitter release from presynaptic nerve terminals (Fig. 1). Tramazoline, methylnorepinephrine and clonidine are examples of relatively selective α_2 agonists. As clonidine also is a potent stimulator of the α receptors which inhibit renin release and lower blood pressure among several other effects, it was held (12) that these α receptors also are of α_2 subtype. This has later been confirmed by the radioactive ligand binding technique (see below). The α_2 receptors are mainly presynaptically located, but some are postsynaptic (42). No simple, generally applicable functional classification of α_1 and α_2 receptors in terms of relative potency of agonists has been developed, and the α receptors are best subclassified according to their affinity for specific antagonists (41, 42, 43), as it will be discussed later on.

As it appears, the adrenergic receptors have been defined, subclassified and studied on the basis of the relative potency of series of sym-

pathetic amines on various physiological effects in intact animals or isolated organ systems. The main advantage of this physiological method is, that it allows direct observation of the physiological effect of the drugs. The method has, however, several drawbacks as regards direct assessment of the receptor affinity, on which the classification and subclassification rest (101):

- 1) The drug must first be transported to its site of action. Therefore, the true drug concentration at the receptor is unknown.

- 2) The drug may bind non-specifically to both extraneuronal and neuronal tissues. The tissues may also take up the drug.

- 3) Some of the responses to the drug may be indirectly mediated.

- 4) The response may undergo desensitization during the course of the experiment as will be discussed in detail later on.

Due to these limitations of the physiological studies to determine the receptor affinity for a particular drug, the development of the radioactive ligand binding techniques in the late half of the seventies has meant a great advance in the study of the adrenergic receptors.

Radioligand binding techniques

The direct radioligand binding studies require highly radioactive and highly specific ligands, a method of separating free ligand from receptor-bound ligand, and criteria and methods for determining the amount of radioligand bound specifically to the receptor in question (8, 41, 42, 43, 101). A ligand is in this context defined as an agonist or an antagonist for an adrenoceptor.

The techniques whereby these ligands are used to study the kinetics and the affinity of drug-receptor interaction have been described in recent articles and reviews (36, 41, 42, 43, 44, 59, 101). In brief, the principles of the methods are as follows:

- 1) A crude cell homogenate is prepared. From this the plasma membrane fraction may be isolated. A few studies have used intact cells instead of homogenates (5, 26, 39, 75, 87).

- 2) The radioligand is added in increasing amounts.

- 3) For each concentration of added ligand the receptor-bound ligand is separated from the free ligand (for example by means of vacuum filtration). Thereafter, the amount of bound ligand is assessed by counting the bound radioactivity.

- 4) In experiments of this type the ligand binds both specifically to the receptor in question and non-specifically to other sites on the membranes, the test tube, etc.

To correct for non-specific binding, the experiment is repeated for each concentration of radioligand in presence of excess of a non-radioactive ligand which is supposed to saturate the receptors. All binding in this second row of experiments must therefore be due to non-specific binding and the amount of specific binding can be calculated as the difference between the total binding and the non-specific binding.

As the receptors can exist in different forms under various conditions and as the binding of a known ligand may vary with the conditions, the specificity of the binding must be tested in each experiment to ensure that the radioligand binds to the desired receptor type or subtype. The criteria for specific binding are (review in (19, 59)):

- 1) The binding must be saturable because the number of receptors per mass unit membrane protein (or per cell) is limited.

- 2) The binding should be of high affinity (as one uses a ligand which should be specific for the receptor type or subtype in question).

- 3) The binding kinetics should resemble very closely the kinetics known from physiological studies of interaction between adrenergic drugs and adrenoceptors. So it should be rapid, reversible and stereoselective.

- 4) The ligand should be displaced by adrenergic drugs in exactly the right order or potency. Thus, for example, isoproterenol should be more potent than epinephrine in displacing ^3H -dihydroalprenolol, which is used as a β selective antagonist. Among the adrenergic antagonists, propanolol, a β selective antagonist, should be much more potent than phentolamine, an α antagonist, in displacing ^3H -dihy-

droalprenolol from the receptor.

The receptor affinity for a radioligand may be assessed in two different ways.

In the first method, which has just been outlined, the radioligand itself is added in increasing amounts until full saturation of the receptors. The results are most often plotted in a Scatchard plot (see (8)). From the Scatchard plot both the total concentration of binding sites (receptor number) and receptor affinity can be determined (a simple mathematical derivation of the Scatchard plot is given in (8)). A linear Scatchard plot means that only one type of receptors exists in the membrane preparation or in the cells in question. Furthermore, a linear Scatchard plot is evidence for non-cooperative ligand-receptor interaction (8), i.e. binding of one ligand to a receptor does not alter the affinity of the receptors in the neighbourhood. A curvilinear Scatchard plot may be interpreted in at least two different ways (8):

1) The curvilinearity may be due to the existence of two classes of receptors. Thus, using an α selective ligand which does not discriminate between α_1 and α_2 receptors (e.g. ^3H -dihydroergokryptine) in a tissue containing both α_1 and α_2 receptors (e.g. uterus) will result in a curvilinear Scatchard plot.

2) The curvilinearity may also result from negative cooperativity. Negative cooperativity means that binding of the first ligand to a receptor with more than one binding site (or linked in some manner to other receptors of the same type) decreases the affinity of the other binding sites on the receptor (or the affinity of some other receptors in the nearest neighbourhood). The insulin receptor seems to have more than one binding site on each receptor molecule and shows negative cooperativity in insulin binding (8). As regards the adrenoceptors, no such cooperativity in drug binding has been found (44). Thus, the curvilinearity of the Scatchard plot in radioligand binding studies of the interaction between adrenergic drugs and adrenoceptors is thought to be due to the existence of more than one type of receptors (e.g. α_1 and α_2 receptors or high-affinity and low-affinity state of α_2 receptors, see below).

The possibility that adrenoceptors show some kind of negative cooperativity in intact cells, where the cytoskeleton (the microtubules) is functioning, has not been definitely ruled out (26).

In the second method, which has been extensively used for subclassification and study of α_1 and α_2 receptors (41, 42, 43, 44) and which also with advantage may be applied for subclassification of the beta receptors (26, 36, 51, 99), increasing amounts of a non-radioactive ligand are added in presence of the radioligand in saturating concentration. In this way the radioligand and the non-radioactive test drug compete for the receptor sites. Taking subclassification of the α receptors as example, the most often used radioligand is ^3H -dihydroergokryptine which binds equally well to α_1 and α_2 receptors (43). To study the ratio of α_1 and α_2 receptors in a given tissue, prazosin may be used as the non-radioactive competitor. Prazosin is a highly selective α_1 antagonist (25), the affinity for α_1 receptors being about 10,000 fold greater than for α_2 receptors (43). The results obtained are plotted in a competition curve (displacement curve), examples shown in (41, 43, 44).

Using a computer modelling technique (43, 44, 57), the proportion of the alpha receptor subtypes can be analysed. The existence of more than one affinity state of a receptor subtype under various conditions (e.g. in the absence of guanine nucleotides (41, 44)) may also be demonstrated with this technique.

This method, where various non-radioactive drugs are used as competitors for a radioactive antagonist, seems to have practical advantages over the first method mentioned (41, 43, 44). The data obtained in this way may be analysed by the Scatchard analysis, but some kind of computer modelling is mathematically to be preferred (28, 36, 57).

The radioligand binding techniques have allowed the adrenoceptors to be quantitated and characterized under various physiological and pathological conditions (59). In the next three passages I shall describe the molecular nature of the β and the α adrenoceptor and the biochemical events following stimulation of these receptors. Our knowledge concerning these

phenomena is far from complete and many results are seemingly contradictory. In this context it must be mentioned that the radioligand binding techniques have several limitations:

1) The techniques give no direct information about the physiological response to receptor stimulation. This fact is especially true when antagonists are used in the study of the receptors. The antagonists have been more widely used because of their higher specific radioactivity, their greater stability and lesser non-specific binding (58).

Therefore, as far as possible, other parameters (e.g. alteration in cyclic AMP or Ca^{2+} -fluxes) should be studied simultaneously with stimulation of the receptors by agonists.

2) The techniques give no information about the subcellular locations of the receptor in question (44, 101).

3) The receptor may be altered by preparation. Most of the studies carried out so far have been done with cell particulates (isolated membrane fractions). Experiments with intact cells (5, 26, 39, 75, 87, 99) have confirmed most of the results obtained with cell homogenates and membrane preparations, but have also pointed out several differences between the behaviour of receptors in intact cells and in homogenates:

a) The number of β receptors decreases when cell metabolism is inhibited. Isolated membranes are devoid of cell metabolism. Cell metabolism may be an important determinant of β adrenoceptor properties (5).

b) The receptor affinity in intact cells has been found to be about one order of magnitude less than the affinity determined in homogenates (26, 75). Furthermore, disruption of the microtubules increases the affinity several-fold (26). The apparent cooperativity is also altered by disruption of the microtubules (26). Several interpretations of this phenomenon are possible (26, 44).

c) One of the events following β receptor stimulation is alteration in the membrane phospholipid metabolism (39, 87). This requires intact cells.

The molecular nature of the β receptor

The β receptor seems to be a lipoprotein. The exact molecular events in the very interaction between drug and receptor are at present unknown, though some speculative models have been proposed (32). The structure-activity relationships for various adrenergic agents have recently been reviewed (32).

The molecular nature of the β receptor and especially its relationship to the enzyme, adenylylate cyclase, which is known to mediate most of the effects of receptor stimulation, has been studied most intensively in the frog and the turkey erythrocyte membranes. From these studies evidence has arisen that the receptor is an entity distinct from the enzyme adenylylate cyclase (97). Vaquelin (97) first succeeded in purification of the β receptor (500–2,000 fold) by affinity chromatography. The receptor was solubilized by treatment of the membranes with the detergent, digitonin. The receptor fraction was fully devoid of adenylylate cyclase activity and it possessed no guanine nucleotide binding sites. This means that the regulatory guanine nucleotide binds to a site different from the receptor molecule itself (see further details below). Furthermore, the β receptor from turkey erythrocytes has been reconstituted by dialysis into human erythrocyte acceptor membranes which themselves lack β receptors and adenylylate cyclase enzymes. The reconstituted beta receptors demonstrated β adrenergic characteristics, but the system lacked adenylylate cyclase activity, confirming β receptors and adenylylate cyclase to be distinct components (46). Recently, β receptors have been solubilized from human placentas (79). The human placenta contains 3–10 times the amount of β receptors found in other human systems and represents an excellent source for further characterization. Wood (102) has succeeded in separation of solubilized β receptors from solubilized α receptors from hepatic plasma membranes. This group concludes that the active β and α adrenergic binding sites do not simultaneously reside on the same macromolecule. As will be discussed later on, this is strong evidence against the adrenoceptor interconversion hypo-

thesis proposed by Szentivanyi and Kunos (53, 54, 91, 92).

Molecular events following β receptor stimulation

It is widely accepted that a rise in the intracellular cyclic AMP (cAMP) concentration due to activation of the adenylate cyclase enzyme follows stimulation of the β receptor. The rise in cAMP concentration has been shown to mediate most, but not all, of the β receptor responses (8, 19, 20, 39, 87). As just mentioned, β receptor and adenylate cyclase are two different membrane components. Therefore, there has been much interest in the coupling between the receptor and adenylate cyclase. A guanine nucleotide sensitive regulatory protein, which is distinct from both receptor and adenylate cyclase, takes part in this coupling (60, 84). In the absence of guanine nucleotides (and in the absence of other purine nucleotides in concentrations greater than 1 mM), the β receptor exists in two different affinity states (58, 60, 84). In the presence of low concentration of guanine nucleotides such as hydrolysable GTP or non-hydrolysable Gpp(NH)p only the low affinity form of the receptor can be demonstrated. On the basis of this and other findings a model for the stimulation of the β receptor by an agonist has been developed (58, 60, 84). This model is illustrated in Fig. 2. The model is described in the legend to this figure.

The rise in the intracellular cAMP concentration mediates most of the effects of the β receptor stimulation; cAMP activating a protein kinase which phosphorylates various regulatory enzymes and other proteins.

Fig. 3 summarizes our present knowledge of the events resulting from stimulation of the β receptor. Apart from the rise in intracellular cAMP concentration, β stimulation also results in activation of the membrane bound catechol-O-methyltransferase (COMT), which is one of the enzymes terminating catecholamine action. The activation of this enzyme is not mediated by cAMP (103). This may be one of the mechanisms whereby catecholamines accelerate their own "clearance" (24).

Stimulation of β receptors also enhances the

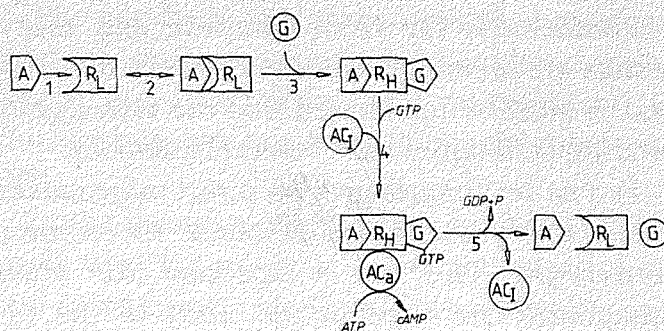


Fig. 2. Molecular mechanisms in activation of adenylate cyclase following β receptor stimulation: In absence of an agonist, the β receptor exists in a low affinity form, R_L [1], which may bind both agonists and antagonists. The agonist A binds reversibly to the low affinity form R_L of the receptor [2]. An antagonist (not shown) also can make this freely reversible binding with the R_L form of the receptor. The agonist-receptor complex then binds some component in the adenylate cyclase system, possibly the guanine nucleotide sensitive regulatory protein, G [3]. This alters the conformation of the receptor to a high affinity state, R_H , which binds the agonist tightly. Also, the G protein becomes able to bind GTP (guanosine triphosphate). Binding of GTP to G [4] results in its association to the inactive adenylate cyclase, AC_I [4] whereby the adenylate cyclase is activated. The active adenylate cyclase, AC_A , catalyses the synthesis of cAMP. The activation of AC and the production of cAMP is terminated when GTP is hydrolysed [5]. Based on data in (42, 58, 60, 84).

methylation of a membrane phospholipid named phosphatidylethanolamine. Phosphatidylethanolamine is methylated to phosphatidylcholine, which is translocated to the outside of the membrane (Fig. 3:2), resulting in greater fluidity (lesser viscosity) of the membrane and hence a more efficient coupling between β receptor and adenylate cyclase (4, 39). The increase of membrane fluidity and the resulting more efficient coupling are possibly partly responsible for the early hypersensitivity following β stimulation (93). This will be discussed in more detail below. This early increase in β receptor sensitivity may also result from unmasking of "cryptic" receptors in the membrane (87). A "cryptic" receptor (48, 98) is a receptor which is preformed and lying in the membrane itself or in some storage near the membrane, and which may be exposed to the outside of the membrane following certain events, e.g. receptor stimulation (Fig. 3:3).

As implied above, the β receptors are subject to alteration in number – and maybe in affinity

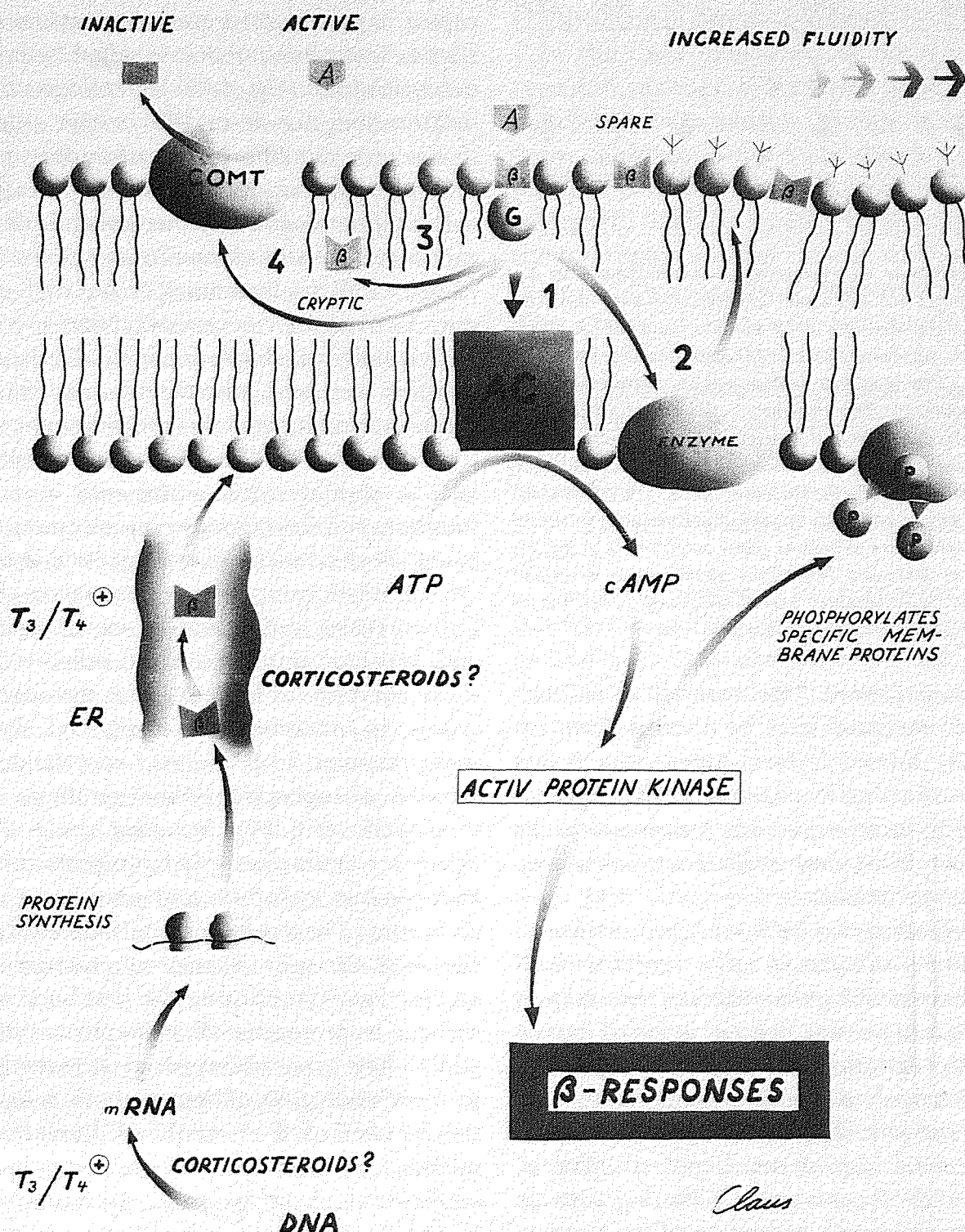
β -STIMULATION

Fig. 3. Molecular events following β stimulation by an agonist. The agonist A stimulates the β receptor whereby the adenylate cyclase AC is activated [1]. The active AC catalyses the formation of cAMP which mediates most of the β receptor effects through activation of protein kinases. Some protein kinase phosphorylates specific membrane proteins whereby the β receptor response to further stimulation may be altered. Stimulation of the β receptor also results in activation of enzymes [2] that enhances phosphorylation of phosphatidylethanolamine to phosphatidylcholine. Phosphatidylcholine is translocated to the outside of the membrane, resulting in greater fluidity of the membrane and hence more efficient coupling between β receptors and AC molecules. β Receptor stimulation also unmasks cryptic receptors [3] and activates COMT which catalyses the breakdown of adrenergic agonists [4]. Furthermore, the figure shows that T_3 and T_4 may increase the β receptor number by increased transport of preformed receptors to the membrane (through the endoplasmic reticulum, ER) or by increased protein synthesis. Corticosteroids may affect the β receptor number in the membrane in similar ways.

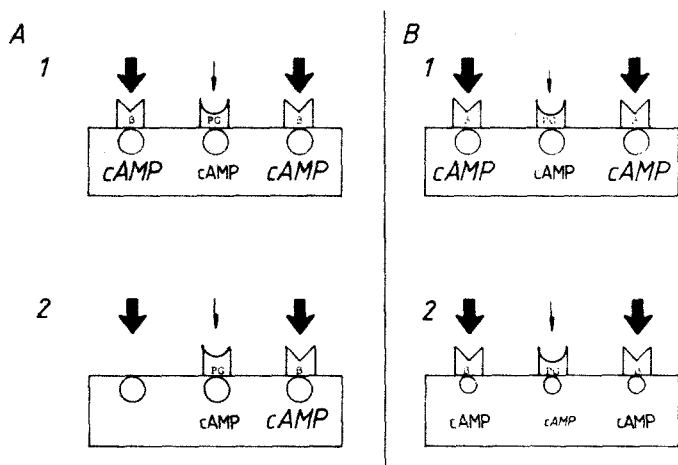


Fig. 4. Homologue and heterologue desensitization. In A₁, the β receptors are stimulated intensely for some time, resulting in a decrease of the β receptor number (A₂) and therefore a decreased total cAMP response to further β stimulation. The adenylate cyclase system, represented by a circle, is unaltered. The response to stimulation of the prostaglandin receptor (PG) is unaffected. This phenomenon is termed homologue desensitization. In B₁, the β receptors are again stimulated intensely for some time. The number of receptors remains constant, but the cAMP response to stimulation of β receptors as well as other receptors (e.g. the PG receptors) is decreased (B₂). This phenomenon is termed heterologue desensitization and is supposed to be due to alteration in the adenylate cyclase system.

— by various factors. The response to stimulation of a receptor may be increased or decreased by several factors. An increase in the response to further stimulation is called upregulation (or hypersensitization). A decrease in the response is called downregulation (desensitization or tachyphylaxis).

Desensitization may be homologue or heterologue. When stimulation of a receptor leads only to decreased responsiveness of the receptor type in question, the process is called homologue desensitization. Thus, when isoproterenol stimulation of the β receptor results in lessened rise in cAMP concentration to further stimulation by isoproterenol, but in unaltered rise in cAMP concentration following stimulation of, for example, a prostaglandin receptor in the same cells, the phenomenon is termed homologue desensitization (Fig. 4,A).

Homologue desensitization is due to a decrease in the total number of receptors (42). Homologue desensitization results in an impaired ability to induce the high affinity state of the receptor and to a decrease in the affinity of the high affinity state of the receptor (51, 99).

When stimulation of a given receptor type results in decreased response not only to further stimulation of the receptor type in question, but also to stimulation of other receptor types coupled to the adenylate cyclase system, the phenomenon is called heterologue desensitization (Fig. 4,B). Heterologue desensitization does not necessarily involve a decrease in receptor number, but is rather due to alteration in the system distal to the receptors (42).

Both homologue and heterologue desensitization require stimulation by an agonist (full or partial) and the formation of the high affinity state form of the receptor (42, 58).

Originally, a simple reciprocal relationship between receptor stimulation and change in receptor binding (number) was thought to exist (review in (48)). Thus, high concentration of agonist should cause a decrease in receptor number, and low agonist concentration should bring about an increase in receptor number.

The relationship between receptor stimulation intensity and duration on the one hand and receptor number on the other is in fact more complex, at least as far as the adrenergic system is concerned. As mentioned above an early response to β receptor stimulation is increased phosphatidylcholine synthesis in the membrane (39, 87), bringing about a more efficient coupling between β receptors and adenylate cyclase molecules and unmasking cryptic receptors. These phenomena may explain the rise in β receptor number on human mononuclear leucocytes during the first hour of continuous isoproterenol or epinephrine infusion, which has been observed in a recently performed study (93). These authors found that the number of β receptors on human mononuclear leucocytes (lymphocytes and monocytes) increased to about two times the baseline value during the first hour of continuous isoproterenol infusion and then fell to approximately half the baseline value after 4 to 6 h of infusion. The alteration in receptor number did not parallel the changes in total mononuclear cell number or in T lymphocyte, B lymphocyte and monocyte distribution. Therefore, the alteration in receptor number must be due to alteration in the number of receptors on the individual cell.

Furthermore, it was shown that the increase in receptor number was accompanied by increased physiological sensitivity to isoproterenol, measured as rise in pulse rate. Thus it may be concluded that the observed increase in receptor number on the peripheral mononuclear cells is paralleled by an increased number of receptors in other cells in the organism. The authors (93) mention that other studies, which have used considerably larger isoproterenol doses, have failed to demonstrate an early increase in β receptor number during continuous infusion of adrenergic substances. The study in question seems, however, to demonstrate that the response to continuous isoproterenol infusion in low to moderate doses are biphasic, showing an early increase and a late decrease in the receptor number.

Persistent stimulation of β receptors in frog erythrocytes has demonstrated a time-dependent selective phosphorylation of different membrane proteins, which may explain why stimulation at one time results in an increase in receptor number, and at another in a decrease in the number (20). However, an early increase in receptor number was not found in this study, but after 16 h incubation the receptor number started to rise again, despite persistent stimulation. *In vivo* studies concerning the long-term effect of adrenergic treatment have not definitely answered the question whether long-term (weeks to months) β stimulation results in a decreased receptor number, but this seems to be the fact (23, 31, 34, 45, 89), as will be discussed in some detail later on.

From the facts just mentioned it follows that the incubation time and the drug dose must be carefully considered when evaluating studies concerning alterations in receptor number during adrenergic therapy.

The same is true when the effects of various factors on the β receptor number are studied. So it is known that both T_3 (triiodothyronine) and T_4 (thyroxine) increase the β receptor number (50, 59). Earlier studies did not reach any conclusion about whether this increase in β receptor number was due to increased protein synthesis or not. The study by Kempson (50) has shown that the increase in β receptor

number following T_3 or T_4 stimulation is independent of protein synthesis in short-term experiments (2–3 h), while the increase in receptor number during long-term experiments (15 h) requires protein synthesis.

In the cases mentioned so far, a change in receptor number in one cell type has been paralleled by a similar change in other cell types. This seems not to be the fact when the effect of corticosteroid on β receptor number is examined. Thus, in rat liver cortisone decreases the β receptor number (59). In rat lung, hydrocortisone increases the β receptor number (62). The effect of corticosteroids on β receptor number thus seems to be tissue dependent. It is at present unknown whether the effect of corticosteroids is mediated by synthesis of new receptors, by increased receptor transport to the membrane, or by unmasking of cryptic receptors. These possibilities do not exclude each other.

The great interest in the adrenergic receptor number in peripheral leucocytes is partly due to the fact that alterations in the receptor number in the circulating leucocytes (and other formed elements of the blood) are thought to reflect alterations in the receptor number in the diseased organ (15, 91). As various subgroups of leucocytes may contain different receptor numbers, there has been a tendency to work with either polymorphs or mononuclear leucocytes or subpopulations of the latter. The human polymorphs contain about 1,800 β_2 receptors per cell (26, 30). The β receptor in human lymphocytes and monocytes seems to be of the β_2 subtype (75, 100), but the β receptor of isolated monocytes does not seem to have been characterized. B and T lymphocytes from peripheral blood seem to bind identical amounts of the β antagonist ^3H -dihydroalprenolol (15). The number of receptors on each cell was not determined in this experiment. Others have found that mononuclear cells from human peripheral blood contain between 1,000 (93) to 2,000 (100) receptors per cell. It is known that the distribution of T lymphocytes, B lymphocytes and monocytes is altered during various disease states and drug treatments (15, 17, 93). Therefore, to be entirely sure that an

eventual alteration in receptor number is due to a true change in the receptor number in each cell and not merely to altered distribution of various subgroups of cells, further work in this field should be done with a pure cell line. The T lymphocyte seems to be favourable in this respect, as it can be isolated fully free of contaminating cells (15).

It has been found (75) that B lymphocytes from human tonsils contain about 600 receptors per cell, while the T tonsil lymphocytes contain only about 200 receptors per cell, some of the latter being without β receptors at all.

The number of β receptors on the mast cell has not been determined. There has been some discussion regarding the type of β receptor on the mast cell, but it seems to have β_2 character (37).

As mentioned in the introduction, the β receptors were originally subclassified on the basis of their organ localization and the relative potency of some sympathetic amines. Thus, it was originally thought that each organ contained only one subtype of β receptor. In the course of time relatively selective β_1 and β_2 agonists and antagonists have been developed, some of which are of great medical importance. The selectivity of these drugs has been based on observations made *in vivo*, *in situ* or in isolated organ preparations (66). Such studies have, as mentioned, several limitations and may be interpreted in different ways. Using these physiological techniques it has often been suggested that some tissues contain a mixture of β_1 and β_2 receptors. This has in turn called in question the true specificity of the known selective β_1 and β_2 drugs. It has been confirmed by direct binding studies that some tissues contain both β_1 and β_2 receptors (36). Rat heart ventricle, an often used model system for β_1 receptors, seems in fact to contain solely β_1 receptors (36). Frog myocardium has about 20% β_1 and 80% β_2 receptors. Rat lung contains 25% β_1 receptors and 75% β_2 receptors (36). Human lung possesses mainly β_2 receptors (6).

Making the assumption that rat heart ventricle has mainly β_1 receptors and rat lung mainly β_2 receptors, the effect of various proposed selective β_1 and β_2 drugs on binding of

the non-selective radioactive β agonist ^{125}I -iodohydroxybenzylpindolol, and on adenylate cyclase activity, were studied *in vitro* (66). This study confirmed the *in vivo* demonstrated specificity of the β_2 agonists, salbutamol, soterenol, feneterol and zinterol, and of the β_1 agonists, practolol and metaprolol. The β_1 specificity of the agonist, dobutamine, and the β_2 specificity of the agonists, terbutaline and metaproterenol could not be reproduced in this study. The authors concluded that the reported *in vivo* specificity of the latter drugs may be due to factors other than receptor specificity (66). However, it must be mentioned that the assumption of a pure β_2 population in rat lung tissue is incorrect, and the lacking *in vitro* specificity of these drugs, whose *in vivo* specificity is well documented, remains to be confirmed.

The conclusion may be drawn that several tissues contain both β_1 and β_2 receptors. In parallel with the α receptors, each β receptor subtype may be separately regulated (41) and individual variation in the amount of each subtype may exist.

The molecular nature of the α receptors

Our knowledge of the molecular events following α_1 and α_2 stimulations is rather sparse. The features of the α_1 and α_2 receptors can be depicted as follows:

The α_1 receptor is typically a postsynaptic receptor which mediates smooth muscle contraction (12). The second messenger mediating the biochemical events following α_1 stimulation is unknown. However, a rise in the intracellular calcium concentration has been suggested as the second messenger mediating the effects of α_1 receptor stimulation (27), see Fig. 5. Doubt exists regarding the origin of the calcium and the mechanism(s) which provides the coupling between the activation of the α_1 receptor and the rise in cytosolic Ca^{2+} concentration (27, 64). In some tissues (27) the main contribution to the rise in cytosolic Ca^{2+} concentration comes from the mitochondria (Fig. 5:6). As the mitochondria are not physically in contact with the plasma membrane, the existence of a messenger which informs the mitochondria about the α_1

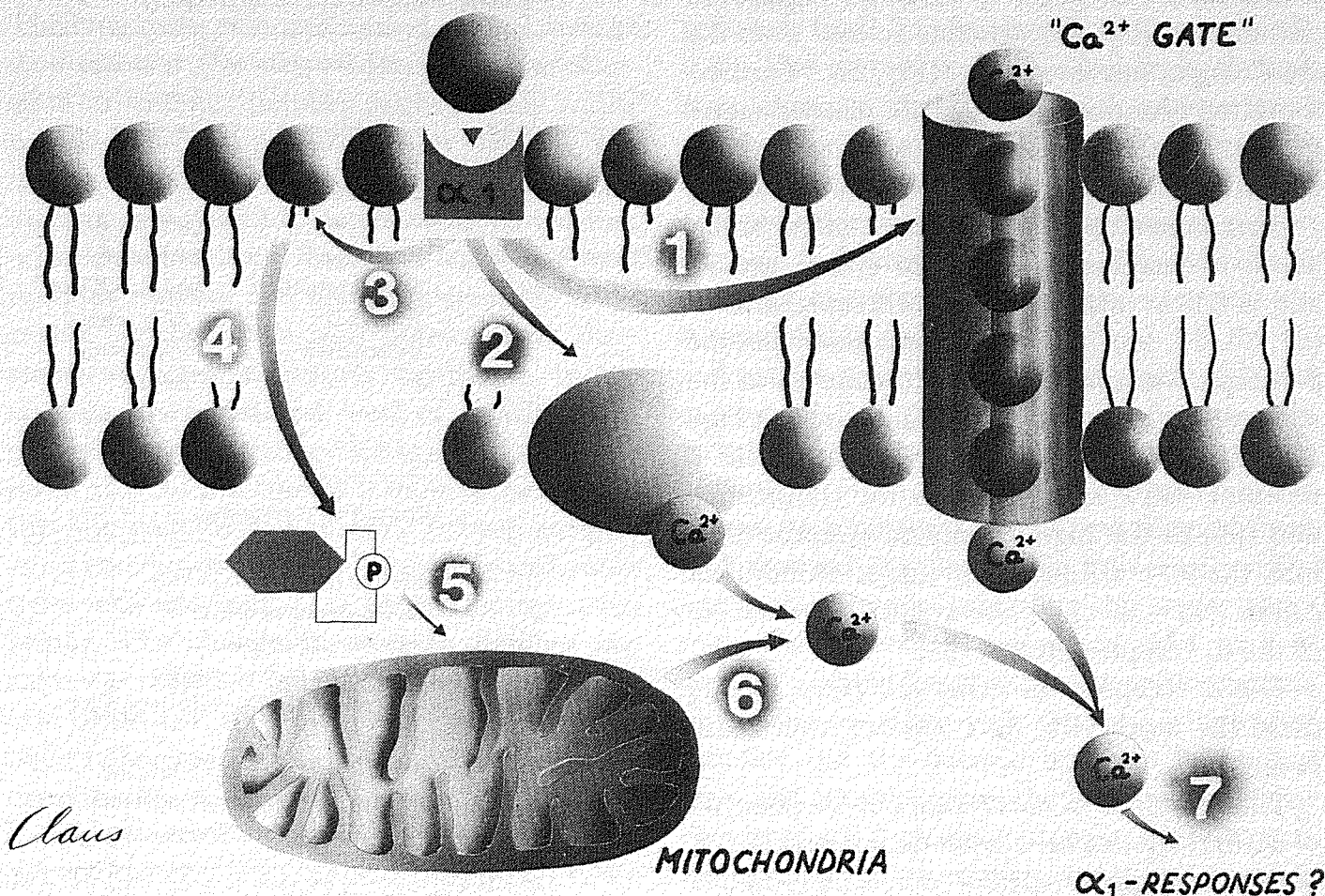
α_1 - STIMULATION

Fig. 5. Molecular events following α_1 receptor stimulation. The true second messenger mediating the responses to α_1 receptor stimulation has not yet been identified with certainty. Ca²⁺ has been suggested as a second messenger for α_1 stimulation. The Ca²⁺ may arise from the extracellular compartment [1], from a Ca²⁺-binding membrane protein [2], or from mitochondria [6]. In the latter case, some intermediary link between receptor stimulation and Ca²⁺ release must exist, possibly in the form of a phosphatidylinositol metabolite [3; 4; 5]. Most α_1 responses require Ca²⁺ [7] and can be mimicked by Ca²⁺ (27).

stimulation at the cell surface has been postulated (27, 64). Stimulation of the α_1 receptor is consistently followed by acceleration of the phosphatidylinositol turnover (Fig. 5:3), and it has been proposed that some metabolite of phosphatidylinositol may be the "true" second messenger (Fig. 5:4) that leads to a rise in cytosolic Ca²⁺ concentration (27). A more simple hypothesis suggests that the rise in cytosolic Ca²⁺ concentration arises from extracellular Ca²⁺ (Fig. 5:1) or from Ca²⁺ bound to some calcium binding protein in the membrane (Fig. 5:2), in which case coupling between receptor stimulation and augmented Ca²⁺ influx in the cytosol may be a more direct event, not necessitating a secondary messenger apart from the Ca²⁺ itself.

Stimulation of α_1 receptors in certain smooth muscles is followed by an increase in intracellular cyclic GMP concentration. This increase in cGMP concentration seems to be secondary to the increase in Ca²⁺ concentration and there is no evidence that cGMP may be the secondary messenger in α_1 stimulation (27).

Human lung contains α_1 receptors (6). In the guinea pig lung, the number of α receptors to β receptors is only 1:16 (7). In human lung, the ratio between α and β binding capacity is 1:6 (92).

The role of α receptors in asthma is discussed in the last section.

The α_2 receptor is typically located presynaptically. α_2 Receptor stimulation inhibits nor-epinephrine release from the presynaptic termi-

nal in the adrenergic nerve system (and thus exerts a sort of negative feedback regulation) and also inhibits acetylcholine release from the cholinergic nerve system (12, 25). Furthermore, α_2 receptors also mediate platelet aggregation, sedation, inhibition of renin release and inhibition of lipolysis in some species (12, 27).

Stimulation of an α_2 receptor often brings about a decrease in adenylate cyclase activity and a fall in intracellular cAMP concentration (27, 33, 78, 96). The α_2 receptor resembles the β receptor in some aspects: In contrast to the α_1 receptor, the α_2 receptor can exist in a high affinity and a low affinity form, just like the β receptor. GTP also promotes the transformation (95) of the high affinity α_2 receptor (α_{2H}) into the low affinity form (α_{2L}). Monovalent cation (Na^+ , K^+ , Li^+) also results in a decrease of the α_2 receptor affinity (33).

The α receptors of many cell types, among these the leucocytes, have not yet been classified.

The existence of α receptors in lymphocytes of several species have been demonstrated (22, 40). In amphibian species, α receptors inhibit secretion of a haemagglutinating substance (40). Stimulation of α receptors in human lymphocytes results in increased activity of lymphocyte ATPase (22). Stimulation of α receptors in mast cells brings about increased histamine release (65).

Table 3 summarizes the known α responses.

The role of the adrenoceptors in atopic diseases

Since Szentivanyi in 1968 (90) proposed that the basal defect in asthma and other atopic diseases is a β adrenergic hyposensitivity or a functional imbalance between β and α adrenoceptors, much work has been done to investigate the true role of the adrenoceptors in atopic diseases, especially asthma. The conflicting results obtained in these numerous studies seem to be due to different approaches to measuring the adrenergic responsiveness and differences in patient populations as regards the severity of diseases, actual disease activity, and medications.

It was first observed that the response to adrenergic stimulation measured *in vivo* as rise

Table 3. Characteristics of α_1 and α_2 receptors		
Subject	α_1 Receptors	α_2 Receptors
Specific agonists	Methoxamine	Clonidine
Specific antagonists	Prazosin	Yohimbine
Affinity forms	Only one	High and low affinity form
Typical location	Postsynaptic	Presynaptic
Major effects	Vasoconstriction	Inhibits NE and ACh release
	Bronchoconstriction	Inhibits renin release
		Increases platelet aggregation
Unclassified α responses		
Glycogen breakdown in liver		
Glucose synthesis in liver		
Inhibition of lipolysis		
Inhibition of insulin release		
Stimulation of salivary K^+ and H_2O secretion		
Inhibition of lymphocyte ATPase		
Alteration of lymphocyte secretion		
Stimulation of histamine release		

in blood lactate, blood sugar, plasma cAMP, urinary cAMP, pulse rate, etc. was diminished among asthmatic individuals (91). *In vitro* studies, in which the rise in cAMP following β adrenergic stimulation in isolated leucocytes was studied, confirmed these *in vivo* observations (91). However, it soon became evident that most of the observed phenomena could be reproduced in normal individuals who had been exposed to adrenergic drugs for some time (23, 31, 49, 73, 91). This raised the question whether the observed adrenoceptor abnormalities seen in asthma, and to some degree in other atopic diseases, were due not to the disease itself but rather to the medication.

The present state of knowledge concerning this topic may be summarized as follows:

Studying human lung tissue removed from patients undergoing thoracotomi for known or suspected lung tumour it was found that the β receptor number is diminished by about 20% and the α receptor number is increased by

about 400% in asthmatics (91, 92) compared with patients without obstructive lung disease. The lung tissue removed looked normal and the data did not give any evidence that the alteration in receptor number might be due to the primary disease process. (It is known that malign tumours may show altered receptor number and types.) In patients with obstructive, non-reversible lung disease a small fall in β receptor number and a small increase in α receptor number were also noted, these alterations were not statistically significant. In the same study, a reduced level of cAMP and a pronounced reduced cAMP/cGMP ratio were noted (52). In this context it must be mentioned that the patients had received 0.6 mg atropine as premedication and adrenergic medications were withheld only 12 h before the operation began. The change in receptor number and ratio was reflected in lymphocytes from peripheral blood (91, 92). In lymphocytes from asthmatics the total binding capacity (the binding capacity for α plus β receptors) was slightly increased and the β/α ratio was reversed from approximately 7 to approximately 1. When lymphocytes from normal individuals were preincubated with adrenergic drugs, the total binding capacity was greatly diminished, but the β/α ratio remained unchanged. Lymphocytes from asthmatics preincubated in the same manner with adrenergic drugs still showed a slightly greater total binding capacity than the preincubated lymphocytes from the normal individuals, but a β/α ratio below unity. These results are very important as they point to a fundamental difference between β adrenergic hyporesponsiveness induced in normal individuals by β adrenergic therapy and the β adrenergic hyporesponsiveness found in asthmatics: In the normal individual exposed to β adrenergic drugs, the total adrenoceptor number is greatly reduced, due to a loss of β receptors which is not compensated by an increase in α receptors. The original β/α ratio, which in human lymphocytes seems to be approximately 7, is conserved. In the asthmatic person, free of β adrenergic medication, the total adrenoceptor number is slightly increased, due to a very great increase in α receptor

number and a less pronounced decrease in β receptor number. The β/α ratio is markedly reduced, from 7 to below unity (approximately 0.8). The asthmatic in β adrenergic therapy may show a slightly reduced adrenoceptor number, compared with a normal person not in adrenergic therapy, but a greater total adrenoceptor number than a normal person exposed to β adrenergic drugs. The β/α ratio in the β adrenergic treated asthmatic remains abnormal (below unity).

Another recently performed study (17) has confirmed that the number of β receptors in lymphocytes from asthmatics is decreased. The patients received no adrenergic medication for at least 48 h before the study and none of them had received any permanent adrenergic agonists in the 2 weeks preceding the study. However, the patients in this study used corticosteroids and/or theophylline. As corticosteroids are known to affect both the total lymphocyte number and the balance between T lymphocytes and B lymphocytes the authors admit that the decreased β receptor number might be due to alteration in the relative amount of T and B lymphocytes. As mentioned earlier, other studies disfavour this interpretation (15, 93). It is of considerable interest to note that a highly statistically significant positive correlation between β receptor number and severity of the asthma (measured as FEV₁/FVC) was found in the study just referred to (17).

Recently, it has been shown in an *in vivo* study (81) that a given rise in pulse pressure and in plasma cAMP requires a greater isoproterenol dose in asthmatics and other atopic individuals (subjects with allergic rhinitis and asymptomatic persons with positive skin tests) than in normal ones. In this study adrenergic medication were omitted for 1 month and theophylline preparations for 2 weeks before the examination.

It has been found that both lymphocytes, granulocytes and isolated epidermal cells from patients with atopic eczema show decreased β adrenergic responsiveness measured as rise in cytosolic cAMP, inhibition of lysosomal enzyme release, glycogenolysis, etc., following β adrenergic stimulation (18, 70, 77). The β receptor number in the granulocytes from patients with

atopic eczema is normal (77) and the response to prostaglandin E_1 , which also stimulates the adenylate cyclase, is also normal (18). Thus, the β hyporesponsiveness in atopic eczema seems to be due to some kind of uncoupling of the β receptors from the adenylate cyclase (77).

As it appears from the above mentioned, there is evidence of a true β adrenergic hyporesponsiveness in bronchial asthma, due to a decreased β receptor number. A number of studies have been done to investigate if the cAMP concentration and/or the rise in cAMP following β adrenergic stimulation are decreased in patients with bronchial asthma. The results have been contradictory. This may be due to several factors:

- 1) The rise in cAMP following isoproterenol stimulation shows an extreme inter-individual (4 fold) and intra-individual (6–7 fold) variation (34), even in normal persons.

- 2) The basal cAMP concentration and the rise in cAMP are influenced not only by β adrenergic hormones and drugs, but also by a lot of other hormones.

- 3) cAMP concentration is increased by drugs that inhibit phosphodiesterase. Many patients in studies of this kind use various theophylline preparations.

- 4) The patient populations studied show great differences with respect to general disease severity, actual disease activity and drug use (34, 49, 67, 68, 73). Often data regarding one or several of these factors are lacking.

Basal cAMP concentration and rise in cAMP following β adrenergic stimulation thus seem to be less suitable parameters for β adrenergic responsiveness in bronchial asthma than direct assessment of the receptor number.

At present, no general conclusions regarding basal cAMP concentration and rise in cAMP concentration following β stimulation in asthmatics can be drawn.

β stimulators, especially β_2 stimulators, are widely used in the treatment of asthma, both as tablets and as inhalators. The question whether long-term use of β adrenergic drugs induces desensitization to further β stimulation has naturally been raised. This important question has at present found no definite answer: Exam-

ining the response to isoproterenol infusion before and during long-term (12 months) terbutaline (5 mg three times daily) treatment of mildly asthmatic patients, Svedmyr (89) found no development of bronchial resistance to further β stimulation, measured as rise in FEV_1 to isoproterenol infusion. Terbutaline was withheld 12 h before the isoproterenol test was performed. At the end of the study, inhalation therapy in the form of terbutaline aerosol 0.5 mg four times daily for 3 days was added to the oral treatment and a new isoproterenol infusion test was done. The patients were then given 1.5 mg terbutaline four times daily as inhalations for 3 additional days and a final isoproterenol test was done. It was found that inhalation therapy improved bronchodilation, measured as FEV_1 before isoproterenol infusion, without causing resistance to isoproterenol stimulation (89).

In contrast to these results, Holgate (45) has found that regularly inhaled salbutamol in doses exceeding 200 μ g four times a day produces progressive loss of airway responsiveness to both salbutamol inhalations and infusions in normal human volunteers. With inhalation of 400 μ g salbutamol four times daily, the β adrenergic resistance develops gradually over 9–10 days.

Examining both asthmatics and chronic bronchitics, Jenne (47) found that continuous terbutaline therapy (5 mg three times daily) causes a small reduction in peak FEV_1 . In this experiment 5 mg terbutaline orally was used as β stimulus after 18 h interruption of the oral maintenance terbutaline therapy. Thus the same β agonist (terbutaline) was used for both the test stimulus and treatment.

The two last-mentioned studies (45, 47) indicate that some degree of β resistance in the airway develops following long-term oral or inhalation β agonist therapy. Following oral terbutaline therapy (5 mg three times a day) the developed bronchial subsensitivity is very slight and may be obscured by elevated baseline values of FEV_1 (47). Jenne mentions (47) that the baseline values of FEV_1 before isoproterenol infusion were elevated at all but one occasion in the study by Svedmyr (89), which may be one

possible explanation why Svedmyr failed to demonstrate development of β resistance in the airways. Another possible explanation is that Svedmyr used one drug for treatment (terbutaline) and another drug (isoproterenol) with different specificity as test stimulus. Furthermore, the inhalation therapy was only given for 6 days, and the results obtained by Holgate indicate that it may take 9–10 days for β resistance in the airways to develop (45).

Thus there is some evidence that long-term oral β agonist (terbutaline) treatment is followed by a slight decrease in β sensitivity in the airways. During normal continuous therapy this seems to be of no clinical importance (47, 89). It may be speculated that this β subsensitivity may assume importance, when inhalations are added to the oral treatment and/or the β agonists are used in great excess (45, 47), but this has in fact not been demonstrated to date.

It may be added that one of the beneficial effects of corticosteroids in the treatment of severe asthma is that these drugs restore the β sensitivity in the airways to normal values (47, 85).

Turning to the role of the α receptors in asthma, it has been demonstrated by use of the radioligand binding technique, that human lungs contain α receptors (6) which are predominantly of the α_1 type. As mentioned earlier, the radioligand binding technique gives no information concerning the subcellular location of the receptors. *In vivo* experiments have, however, provided indirect evidence that some of these α_1 receptors mediate bronchoconstriction and therefore must be located in the bronchial smooth muscle cells (see below). The role of the α receptors in bronchial asthma may be summarized as follows:

Although a few relatively recent studies (76, 86) question the existence of α receptor in human airways or their role in bronchial asthma, the bulk of evidence favours their existence and gives them a definite role in mediating bronchoconstriction. Thus it has been found (72) that phenylephrine, a potent α agonist, causes significant bronchoconstriction (measured as fall in FEV₁ and specific airway conductance) in patients with extrinsic asthma

after prior β blockade with propranolol. This bronchoconstricting effect of phenylephrine could be abolished by α blocking drugs such as phenoxybenzamine and thymoxamine.

Various α antagonists (indoramine, phentolamine, thymoxamine) have been found to inhibit bronchoconstriction under certain circumstances and to be of value in treatment of certain asthmatics, although no double blind clinical test of α blockers in the treatment of asthma in general seems to have been conducted:

Indoramine (14), phentolamine (11) and thymoxamine (71) all give significant protection against exercise-induced bronchoconstriction in asthmatics. In the case of indoramine, it has been demonstrated that this effect is not due to the antihistaminic properties of the drug (14). Thymoxamine is almost completely devoid of antihistaminic properties (13) and its inhibitory effect on exercise-induced asthma is equal to that of sodium cromoglycate (71). Furthermore, thymoxamine also significantly inhibits histamine-induced bronchoconstriction in both normal individuals (13) and extrinsic asthmatics (29) and allergen-induced bronchoconstriction in extrinsic asthmatics (74). It has been suggested that the main effect of thymoxamine is in the bronchomotor tone rather than on mediator release (74).

Using a double blind protocol, phentolamine was found to block exercise-induced asthma in a single patient, in whom conventional therapy had failed to reduce the exercise-induced asthma attacks (35).

Black (16) has investigated the long-term effect of indoramine in eight patients suffering from both asthma and migraine. The patients were told that indoramine was prescribed for migraine prophylaxis. This experiment may therefore be regarded as "single blind". Three of the eight patients experienced a subjective improvement in their asthma and their airflow-meter readings increased significantly. Therefore, it may be concluded that α blockers are of value in the treatment of at least some asthmatics. Furthermore, using the capacity of phenylephrine to constrict the cutaneous vascular bed and to dilate the pupil as a measure of α

sensitivity, Henderson (38) found that asthmatics show α adrenergic hyperresponsiveness when compared with normal and patients with allergic rhinitis. This finding has been confirmed by others (80).

The mechanisms whereby the α hyperresponsiveness induces or potentiates asthma have not been fully elucidated. However, it has been found that leucocytes of asthmatics show increased ATPase activity (21) and α stimulation increases the ATPase activity (22). As α stimulation also inhibits the rise in intracellular cAMP concentration (73), it has been speculated that the increased ATPase activity depletes the bronchial smooth muscle cell of ATP, whereby the formation of cAMP is hindered. The inhibiting effect of α stimulation on cAMP formation may, however, as well be due to a more direct effect on the adenylate cyclase system. It has been shown in several *in vitro* studies that α blockers restore the β sensitivity in leucocytes from asthmatics towards normal (3, 61, 73). It should be mentioned that corticosteroids also inhibit leucocyte ATPase (21) and α receptor-induced bronchoconstriction (94) which may suggest a partly common mechanism of action of corticosteroids and α blockers (73). Regarding the importance of the α adrenergic hyperresponsiveness in asthma it is added that bacterial endotoxin has been found to increase the α responsiveness several fold (82). Therefore, α blockers may be of special value in treatment of asthma induced by bronchial infection (63, 82).

In conclusion it may be said that patients with bronchial asthma show a β adrenergic hypo-responsiveness and an α adrenergic hyperresponsiveness. It has been speculated that this fact is due to a β to α adrenoceptor interconversion (2, 53, 54, 69, 91), but no true evidence for this theory has at present been obtained (2, 9, 10) and some experiments give strong evidence against the adrenoceptor interconversion theory (9, 10, 102).

The value and efficacy of β agonists in asthma therapy is well known and need no further consideration.

On the basis of the facts given above, it may be thought that α blockers will be of value in the treatment of selected groups of asthmatics

and types of asthma attacks: (i) corticosteroid dependent asthmatics, (ii) asthmatics with airway hyperreactivity to inhaled histamine, (iii) exercise-induced asthma, and (iv) asthma induced by bronchial infection. In these cases, α antagonists may be of value as a supplement to β agonist therapy, rather than a substitute for the latter. As the α receptors in human lung are mainly of the α_1 type, future clinically controlled trials are probably best done with a selective α_1 antagonist (e.g. prazosin). Phentolamine, which is a non-selective α antagonist, blocking both α_1 and α_2 receptors, has been reported to cause bronchoconstriction in some patients (83). This was to be expected, as blocking of α_2 receptors may result in enhanced cholinergic activity. In small infants the use of α blockers is not recommended, as the α receptor mediated reduction in mucosa oedema may be of great value in these patients (88).

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