unlike many hormone receptors, earlier intensive classical pharmacological studies have led to the development of a multitude of pharmacological agents that have been crucial in our understanding of the nature of hormone and neurotransmitter receptors and their association with the membrane-bound enzyme adenylate cyclase. Much emphasis has been placed on α- and, in particular, β-adrenoceptors, since, unlike many hormone receptors, earlier intensive classical pharmacological studies have led to the development of a wide range of pharmacological agents that have been crucial in the characterization of adrenoceptor subtypes and in the mechanism of receptor–effector coupling. For example, the availability of selective competitive antagonists, full and partial agonists, has proved critical in the dissection of components involved in the coupling of these adrenoceptors to adenylate cyclase and in the regulation of this transmembrane signalling process.

It is now clear that the β-adrenoceptor complex consists of a number of separate, though interacting, protein entities (receptor recognition site, one or possibly more guanine nucleotide-binding proteins (N-proteins or G-proteins) and the adenylate cyclase unit (for review, see Ross & Gilman, 1980: Limbird, 1981). The crucial role of guanine nucleotides in transferring information from the receptor to adenylate cyclase is now widely recognized (Limbird, 1981) and it now seems likely that alterations in the efficiency of this receptor–effector coupling may be an important mechanism by which cells regulate their responsiveness to catecholamines (Su et al., 1980; Iyengar et al., 1981). Although the precise mechanisms of β-catecholamine-induced stimulation of adenylate cyclase are not fully understood, it is widely accepted that binding of an agonist to the receptor promotes the exchange of GDP by GTP at the G-protein and that the GTP bound form of the protein activates the cyclase. Activation of the enzyme is turned off by the hydrolysis of bound GTP to GDP and Pi by GTPase activity associated with the G-protein (Cassel et al., 1977). There is much evidence compatible with this mechanism; for example, non-hydrolysable GTP analogues can cause persistent activation of cyclase (Londos et al., 1974), and cholera toxin, which greatly enhances GTP-induced activation of the cyclase, has been shown to inhibit GTPase activity in turkey erythrocytes (Cassel & Selinger, 1977) possibly by ADP-ribosylation of components of the G-protein (Johnson et al., 1978).

More recently, on the basis of the above evidence and on the behaviour of β-adrenoceptor agonists and antagonists in receptor binding assays, a ternary complex model of β-adrenoceptor–cyclase coupling has been proposed (Kent et al., 1980; De Lean et al., 1980). In this model it is believed that agonists induce or stabilize a complex of agonist, receptor and G-protein and that generation of this ternary complex is an absolute pre-requisite for cyclase activation. Further it seems probable that the extent by which an agonist induces or stabilizes the ternary complex relates directly to the relative efficacy of the agonist in terms of cyclase activity, and there is direct evidence that the G-protein that induces high-affinity binding of agonists to the β-receptor also conveys nucleotide-dependent stimulation to the catalytic unit (Stadel et al., 1981). In practical terms within a ligand binding assay, the potency of agonists to promote the formation of a receptor–G-protein complex corresponds to the ability of the agonist to form a high-affinity state of the receptor and is the basis for the shallow displacement curves that are characteristic of agonists competing against labelled antagonist ligands. Predictably, addition of guanine nucleotides to the assay results in a shift of the agonist competition curve to the right with steepening. This corresponds to the ability of guanine nucleotides to dissociate the receptor–G-protein complex resulting in a homogeneous population of receptors with lower affinity for agonists.

It should, however, be emphasized that this ternary complex model has been largely derived from data obtained from frog and avian erythrocytes and that there is evidence that these β-adrenoceptor systems may differ from those found in mammalian tissues. For example, we have recently provided evidence that the pharmacological characteristics of frog or chick erythrocytes do not strictly correspond to either the β1 or β2 subtypes associated with mammalian tissues (Dickinson & Nahorski, 1981a). Moreover, extensive studies of the pharmacological characteristics of β-adrenoceptor binding sites in several mammalian tissues have revealed the co-existence of β1 and β2-adrenoceptors in many tissues (for review, see Nahorski, 1981). Since there is increasing evidence that both subtypes may be present on the same cell and mediate, under certain circumstances, the same physiological function (O’Donnell & Wanstall, 1981), it would seem critical to evaluate whether both subtypes display similar mechanisms of coupling to adenylate cyclase.

Previous work from this laboratory has established that the non-subtype selective β-adrenoceptor antagonist I'Hidroalpranolol specifically labels sites in rat and rabbit lung that possess properties indicative of an interaction with β-adrenoceptors. Moreover, the use of highly selective β1- and β2-agonists and computer-assisted curve fitting has established that, whereas rat lung possesses predominantly β2 receptors, rabbit lung possesses predominantly β1 receptors (Rugg et al., 1978; Dickinson & Nahorski, 1981b). Examination of the effect of GTP on β2-rich rat lung membranes revealed the agonist-specific effects of this nucleotide. Thus the occupation curve of the antagonist propranolol had a slope factor of close to unity and was totally unaltered by GTP. Salbutamol, which is a partial agonist at rat lung adenylate cyclase (Maximum stimulation 56% of that of the full agonist isoprenaline), generated a displacement curve of low overall slope (slope factor, nH = 0.8) in the absence of GTP. GTP decreased the overall IC50 (concentration producing 50% inhibition) of salbutamol by 2-fold and in the presence of the nucleotide the slope of the occupation curve approached unity. The full agonist isoprenaline was markedly affected by GTP, resulting in a 3.3-fold decrease in overall affinity and steepening of the displacement curve (Fig. 1). The binding data were subjected to
computer-assisted iterative curve fitting to a one- or two-affinity-state model. In the absence of GTP the curve can be well fitted to two (high and low) affinity state model, whereas in the presence of the nucleotide the data was best fitted to a single low-affinity state. These data suggest that mammalian \( \beta_1 \)-adrenoceptors, like those previously examined in frog erythrocytes (Kent et al., 1980), correspond to a ternary complex model in which GTP interconverts high-affinity sites to low-affinity sites.

However, data obtained from the \( \beta_1 \)-rich lung (Fig. 2a) suggested that under conditions identical with those used in rat lung (50 mM-Tris buffer, pH 7.8, in the absence of bivalent cations at 22°C), isoprenaline generated curves of low slope (nH = 0.78) that were only slightly shifted to the right by GTP with no effect on the slope. Since rabbit lung contains both \( \beta_1 \) and \( \beta_2 \)-adrenoceptors in a ratio of 4:1, GTP effects on agonist binding could be difficult to interpret. The subtypes were therefore isolated by suppressing binding to \( \beta_2 \) or \( \beta_3 \)-sites with highly selective antagonists (Fig. 2b). Isoprenaline binding to the isolated \( \beta_1 \)-sites could now be shown to be not influenced at all by GTP, whereas the curve of isoprenaline binding to the isolated \( \beta_3 \)-sites showed the characteristic steepening and shift to the right in the presence of the nucleotide.

A number of different conditions of incubation and membrane preparation were attempted in order to reveal guanine nucleotide sensitivity at \( \beta_1 \)-adrenoceptors, and, in agreement with other reports that the 'pseudo' \( \beta_1 \)-adrenoceptor-effector coupling in
turkey erythrocytes was markedly temperature- and Mg\(^{2+}\) dependent (Simpson & Pfeuffer, 1980; Shane et al., 1981), we were able to show that the "isolated" β1-sites in rabbit lung, in complete contrast with the situation at 22°C, display guanine nucleotide-sensitive agonist binding at 37°C in the presence of Mg\(^{2+}\) (Fig. 2f). These results suggest that, under certain conditions, β1- and β2-adrenoceptors have, even within the same tissue, differential abilities to form high-affinity GTP-sensitive agonist-receptor complexes. Since it is this high-affinity state that may be the required intermediate for activation of cyclase, these findings suggest that β1- and β2-receptors may be differentially coupled to the enzyme. In view of the clear differential effect of Mg\(^{2+}\) in β1- and β2-adrenoceptor coupling, one might speculate that the effects of this bivalent cation may relate to: (1) occupation of cation-binding sites that may allosterically alter the affinity of GTP for the G-protein; (2) the production of Mg\(^{2+}\).GTP, which could be a more effective substrate for G-protein; or (3) an Mg\(^{2+}\)-enhanced rate of agonist-stimulated dissociation of GDP from the protein.

The differential requirement of Mg\(^{2+}\) and dependence on temperature at β1- and β2-sites could also suggest that different G-proteins are associated with each receptor subtype. In this respect, it is of interest that recent analysis of the subunit structure of the avian erythrocyte (β1-like) G-protein has revealed the absence of the 52,000-mol.wt. subunit in this system (Sternweis et al., 1981). It will be important to establish whether this is equally true for mammalian β1-adrenoceptors and whether this relates to the less efficient coupling at this receptor subtype.

The significance of these findings in physiological terms remains to be established, though they could underly the mechanism by which partial agonists such as salbutamol and totenol display selectivity towards β1-adrenoceptors without possessing selective affinity for that receptor subtype (Nahorski, 1979). In our hands, salbutamol displays little if any activation of cyclase at β1-receptors in rabbit lung or in avian erythrocytes. Likewise, salbutamol occupation curves in binding assays at these receptors do not display GTP sensitivity even under optimal incubation conditions.

### α-Adrenoceptors

The sub-classification of α-adrenoceptors into α1 and α2 has been made on pharmacological grounds in view of the differential affinities of various agonists and antagonists in various systems (Stark & Docherty, 1980). It now seems probable that this receptor differentiation can also be made on the basis of the respective effector systems for the subtypes. Thus, only α1-adrenoceptors appear to be negatively coupled to adenylate cyclase, whereas α2-adrenoceptors may alter Ca\(^{2+}\) fluxes by stimulating phosphatidylinositol turnover (Berridge, 1981).

Unlike the situation with β-adrenoceptor-stimulated adenylate cyclase, the mechanisms by which α1-adrenoceptors reduce the activity of the enzyme is much less clearly understood. However, it has been established that GTP is also essential for inhibition of adenylate cyclase by many agents, including α-agonists (Jakobs, 1979). Moreover, in contrast with stimulation of adenylate cyclase, stable GTP analogues reverse or suppress hormone-induced inhibition of adenylate cyclase, and recent studies (Aktories & Jakobs, 1981) have suggested that α-agonists may accelerate the turn-off reaction by stimulating the activity of GTPase.

The recent availability of specific α1-antagonist ligands ([\(^{3} \)H]yohimbine and [\(^{3} \)H]rauwolscine has allowed us and other groups to probe the interactions of guanine nucleotides with agonist binding to α1-adrenoceptors. In human platelet lysates, which represent a rich source of α1-adrenoceptors linked negatively to adenylate cyclase, ligand-binding studies indicate remarkable similarities to that at stimulatory systems such as β adrenoceptors. Thus, agonists and, to a lesser extent, partial agonists generate shallow displacement curves against [\(^{3} \)H]yohimbine binding in the absence of guanine nucleotides. Addition of GTP shifts the curve to the right with a steepening so that slopes closely approach unity (Barnett et al., 1982; Hoffman et al., 1982). These properties, which are not seen with antagonists, are consistent with the presence of two affinity states of the α1-adrenoceptor, and suggest that a similar high-affinity ternary complex may also relate to inhibition of adenylate cyclase. Although present evidence suggests that the G-protein associated with inhibition of the enzyme differs from that related to stimulation (Cooper, 1982), investigation of the complex interplay between stimulatory β1- and β2-adrenoceptors and inhibitory α1-adrenoceptors in a single cell type such as the hamster adipocyte should reveal new information concerning the regulation of cellular function by the endogenous catecholamines noradrenaline and adrenaline.

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