Br. J. clin. Pharmac. (1991), **31**, 501–514 ADONIS 030652519100096U

THE 1990 LILLY PRIZE LECTURE

A way of looking at agonism and antagonism: Lessons from salbutamol, salmeterol and other β-adrenoceptor agonists

DAVID JACK
Former Research and Development Director, Glaxo Holdings Limited, Ware, Hertfordshire

Introduction

The award of the Lilly Prize for clinical pharmacology to a non-medical industrial scientist must have surprised many people. In my case, however, surprise soon turned to pleasure as I realised that it was not primarily a personal award but recognition of the contributions to therapeutics made during the past 25 years by the research group in Allen & Hanbury's (now Glaxo Group Research) laboratories at Ware. Another pleasing feature was that the award recognised the importance of the interface between industrial pharmaceutical research and clinical pharmacology for drug discovery and development. Indeed, its significance is so great that I considered making it the subject of this paper. Fortunately for all of us, perhaps, your Chairman preferred my alternative offer of a presentation of new adrenergic pharmacology.

My purpose is to outline the theoretical and practical consequences of a way of thinking which I developed after the discovery of salbutamol in 1966 in order to explain its unexpectedly high selectivity of action at β_2-adrenoceptors, and used through the years to assess and occasionally make research proposals. The subtitle of the paper reflects its main themes. In the first theme the determinants of the potency of β-adrenoceptor agonists are defined and how this led to the concept of Type 1 and Type 2 agonism is outlined. The second is a brief account of the discovery of salmeterol and an explanation of its exceptional pharmacological properties. The third is a demonstration that the variety of interactions which occur between β-adrenoceptor agonists and their receptor proteins is much greater than is generally supposed. However, before attempting a pharmacological journey of this kind, it is essential to have a clear understanding of the physiology on which it is based. I begin, therefore, by considering the mechanism of action of adrenaline at β-adrenoceptor proteins because this is the key to all β-adrenergic pharmacology.

The action of adrenaline at β-adrenoceptors

The engagement of adrenaline with β-adrenoceptors on the cell surface has long been known to be followed by increased intracellular levels of cyclic-3′,5′-adenosine monophosphate (cyclicAMP) (Robison *et al.*, 1967; Sutherland & Rall, 1960) but only during the past 10 years have the intervening steps involving G-proteins in this and other processes been clarified (Gilman, 1987). The process is complicated and not yet fully understood, but the general principles of how adrenaline controls the G-protein cycle and subsequent cellular events seem clear.

My understanding of how adrenaline induces β-adrenergic events is illustrated and explained in Figure 1, which is based on a simplified version of Birnbaumer's model (Birnbaumer *et al.*, 1990). I consider the β-adrenoceptor protein to be an enzyme whose ground state (R^o) is converted into the active adrenaline-receptor protein complex (AdR^{Ad}) by a conformational change induced when it combines with adrenaline (Ad). The adrenaline binds at the physiological β-adrenoceptor which, given the relative sizes of the mediator (Da 183) and the protein (kDa > 60), must be a relatively small assembly of groups in the protein. Furthermore, because adrenaline is a polar molecule, its receptors are probably located on or near the outer polar surface of the protein.

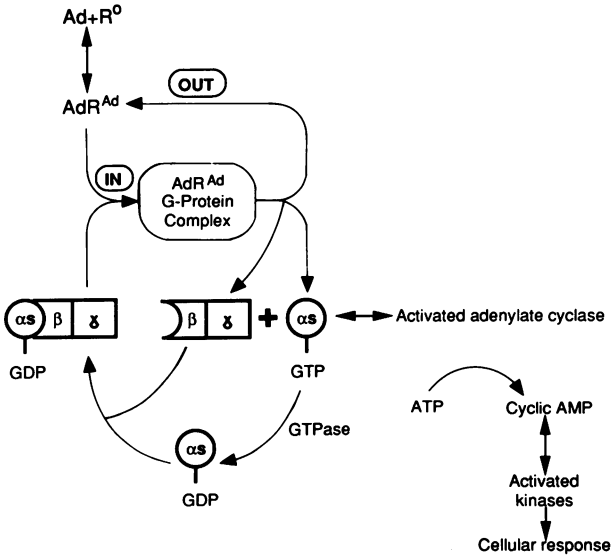


Figure 1 Diagram showing how adrenaline (Ad) reacts with the β-adrenoceptor protein (R^o) to form the activated enzyme ($Ad\ R^{Ad}$) which catalyses the formation of α_s-GTP from a trimeric G_s protein (α β γ). α_s-GTP activates GDP adenylate cyclase and the cellular response is continued. The effect of adrenaline ceases when $Ad\ R^{Ad}$ dissociates and that of α_s-GTP when it disengages from its receptors and/or is hydrolysed to α_s-GDP because of its intrinsic GTP-ase activity. While they exist each $Ad\ R^{Ad}$ complex and each α_s-GTP-cyclase complex process a number of their substrate molecules and so low concentrations of extracellular adrenaline give rise to higher concentrations of cyclic-AMP inside the cell.

The AdR^{Ad} complex contains on its inner surface an active centre which binds and processes its substrate, a specific trimeric G-protein. The end-products of enzyme action are α_s-GTP (formed from the α_s-GDP unit of protein by exchange of GTP for GDP), the $\beta\gamma$-residue of the protein, and unchanged AdR^{Ad}. How α_s-GTP continues the cellular response by activating adenylate cyclase and how the G-protein is regenerated are also illustrated in Figure 1. For the present purpose, however, the important features of the mechanisms depicted are the following:

1. Adrenaline enters the G-protein cycle as the active complex AdR^{Ad} and leaves it in the same form (IN and OUT in Figure 1). The role of AdR^{Ad} is, therefore, catalytic and the receptor protein may be regarded as an enzyme whose activator is adrenaline and whose substrate is G-protein.

2. Each regenerated molecule of AdR^{Ad} is free to take part in another G protein cycle *or* to dissociate into Ad and R^o. The former is more likely because AdR^{Ad} is moderately stable and G-protein molecules are readily available. On average, therefore, each AdR^{Ad} complex catalyses the formation of a number of α_s-GTP molecules.

3. The biological activity of adrenaline is determined by its ability to generate α_s-GTP; all subsequent events in the cellular response are 'down-stream'.

4. The rate of generation of α_s-GTP is determined (i) by the time taken to bind and process each G-protein molecule and (ii) by the stability of AdR^{Ad}, since this determines the average number of G-protein cycles catalysed by each AdR^{Ad} complex. (i) corresponds to pharmacological efficacy (Stephenson, 1956); (ii) is determined by the energy of activation for the process $AdR^{Ad} \rightarrow Ad + R^o$.

Determinants of the potency of β-adrenoceptor agonists

If the deductions in the preceding section are right an unnatural β-adrenoceptor agonist will be more potent than adrenaline if it forms a more stable complex with the β-adrenoceptor protein and/or a complex of greater catalytic activity. Similarly, it will be less potent than adrenaline if its receptor-protein complex is less stable and/or of lesser catalytic activity than the corresponding adrenaline complex.

These are exactly the conclusions we reached many years ago by interpreting structure-activity relationships in β-adrenoceptor agonists (Brittain *et al.*, 1970, 1976; Jack, 1977). For example, it is obvious from the data in Table 1 that in the catechol series, agonists more potent than adrenaline at β_2-adrenoceptors are obtained simply by replacing its *N*-methyl group by larger non-polar substituents and that noradrenaline, which lacks an *N*-methyl group, is less active. The reason for this is that the fit of the larger catecholamines at the flexible receptor protein involves additional interactions as illustrated in Figure 2. In consequence, their affinities for their receptors are greater and the resultant more stable

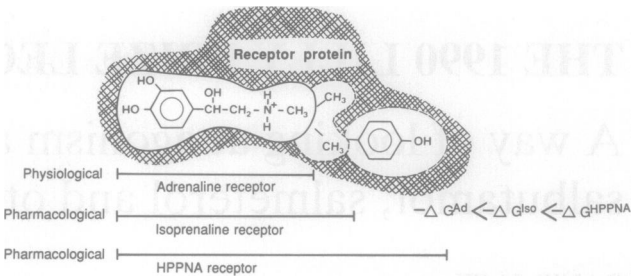


Figure 2 Diagram to show differences between the receptors for adrenaline, isoprenaline and *p*-hydroxyphenylisopropylnoradrenaline (HPPNA) within the β-adrenoceptor protein. The decreases in free energy associated with their combination with the receptor protein are ΔG^{Ad}, ΔG^{Iso} and ΔG^{HPPNA} respectively.

agonist-receptor protein complexes process more substrate molecules than the adrenaline complex during their life-times. Noradrenaline is less active because it forms a less stable complex with the receptor protein.

The cause of the parallel increasing β_2-adrenoceptor activity in the saligenins is similar but they are all less active than the corresponding catechols because their efficacies are lower.

The catecholamines and their saligenin analogues are not special cases because similar trends in potency at β_2-adrenoceptors with increasing molecular size occur in all other series of analogues of adrenaline (Brittain *et al.*, 1976). The potencies of individual β-adrenoceptor agonists have also been found to correlate well with their affinities for β_1-receptors (Bilizikian *et al.*, 1977) and β_2-receptors (Minneman *et al.*, 1979). β-adrenoceptor agonists which form more stable AgR^{Ag} complexes than adrenaline are, therefore, commonplace. However, agonists which form more efficacious complexes than adrenaline are rare, the only candidates known to the author being the carbostyril derivatives whose exceptional activity at β_2-adrenoceptors is shown in Table 2.

Our 1970s model for β-adrenoceptor agonism (Jack, 1977), illustrated in Figure 3, is simply another statement of occupation theory of drug action. It assumes that each agonist (Ag) forms a unique complex (AgR^{Ag}) by binding to its pharmacological receptors in the receptor protein (R^o). Such complexes vary in stability according to the decrease in free energy ($-\Delta G$) associated with the

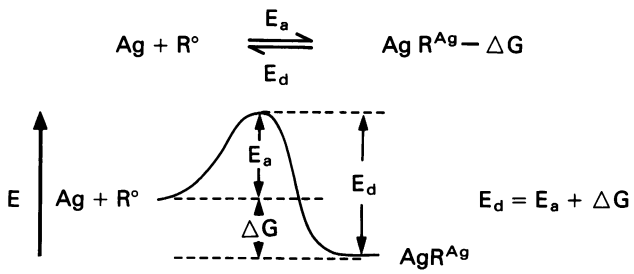
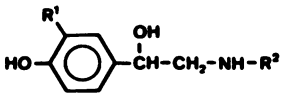
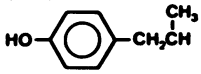
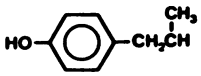


Figure 3 Essential thermodynamic relationships for a reversible bimolecular reaction between a β-adrenoceptor agonist (Ag) and a β-receptor protein (R^o) to form an activated complex AgR^{Ag}. E_a and E_d, the energies of activation for the association and dissociation processes respectively, determine their rates and the rate of attainment of the steady state. ΔG, the decrease in free energy associated with the formation of AgR^{Ag}, determines the position of the steady state.

Table 1 Relative activities and affinities of selected catecholamines and their saligenin analogues at preparations containing β_1- or β_2-adrenoceptors

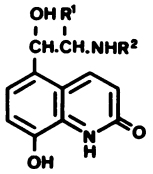


Catechols R^1 = − OH
Saligenins R^1 = CH_2OH

		Relative activity Equipotent concentration; (−)-isoprenaline = 1		*Relative affinity (−)-isoprenaline = 1*	
Compound	*R^2*	*guinea-pig tracheal muscle*[1] *(β_2-adrenoceptors)*	*guinea-pig left atria*[2] *(β_1-adrenoceptors)*	*rat lung membrane*[3] *(β_2-adrenoceptors)*	*rat left atrial membrane*[3] *(β_1-adrenoceptors)*
Catechols					
(−) Noradrenaline	H-	56	10	80	14
(−) Adrenaline	CH_3-	5	12	6	10
(−) Isoprenaline	$(CH_3)_2CH$-	1 (pEC_{50} = 7.7)[a]	1 (pEC_{50} = 7.9)[a]	1 (pK_i = 6.71)[a]	1 (pK_i = 6.52)[a]
(±) *t*-Butylnoradrenaline	$(CH_3)_3$-C-	0.4	3.6		
(±) *p*-Hydroxyphenylisopropyl-noradrenaline		0.1	0.5		
Saligenins					
(±) AH3364	H	580	10,000		
(±) AH4053	CH_3-	430	3500		
(±) AH3021	$(CH_3)_2CH$-	6	> 1000[b]		
(±) Salbutamol	$(CH_3)_3C$-	3.7	> 2000[b]	10	26
(±) AH4553		1.3	> 2000[b]		

[1] Inhibition of induced tone (Coleman & Farmer, 1971).
[2] Electrically stimulated inotropic response (Coleman & Nials, 1986).
[3] Competitive binding *vs* [^{125}I]-(−) iodopindolol.
[a] pEC_{50} and pK_i are the negative logarithms of the concentration producing half maximal response and of the equilibrium constant respectively.
[b] partial agonist.

Table 2 The β-adrenoceptor stimulant activities of variously substituted 5[1 hydroxy-2-alkyl-amino-alkyl]-8-hydroxy carbostyrils (from Yoshizaki *et al.*, 1976)



		Relative equipotent concentration (−)-isoprenaline = 1	
R^1	R^2	*Relaxation of tracheabronchial muscle*[1]	*Cardiac stimulation (rate)*[2]
H	H	4	1827
H	$CH(CH_3)_2$	0.00005	810
H	$CH_2CH(CH_3)_2$	0.0003	16
H	$C(CH_3)_3$	0.0004	23
H	CH_2Ph	0.07	3468
H	$C(CH_3)_2CH_2Ph$	0.001	0.001

[1] Guinea-pig isolated tracheal chain.
[2] Guinea-pig isolated right atria.

binding process, and in catalytic activity (efficacy) depending on how perfect an active centre is formed in the agonist-receptor protein complex. The rate of onset of action of an agonist is determined by its concentration and the energy of activation (E_a) for its association with its receptors, which determine the rate of formation of active complexes, and by its efficacy. The potency of an agonist, under steady state conditions, is determined by its affinity for its receptors, ($-\Delta G$) and its efficacy. The rate of offset of action, after removal of the agonist, is determined by the energy of activation (E_d) for its dissociation from its receptors.[1]

This model was a useful working guide but is obviously over simple. For example, by assuming that R^{Ag} reverts rapidly to R^o when Ag dissociates, free energy and entropy changes associated with conformational changes in the receptor protein are ignored. Similarly, no account is taken of the relative contributions of changes in heat content (ΔH) and entropy (ΔS) to the ΔG value determined. Serious analysis of such changes is beyond the scope of this paper and, in truth, beyond that of the author as well. I mention these factors now only because, as shown later, the tertiary structures of the activated

[1] A partial agonist behaves like a full agonist except that its efficacy is so low that it is incapable of inducing a maximal cellular response at any concentration. It antagonises the effects of a full agonist. A pure isosteric antagonist has zero efficacy because it disorders the receptor protein. Its potency is determined by its affinity for its receptors; its pA_2 value is a function of $-\Delta G$ (ΔG = 2.303 RT log pA_2). Its onset and offset of action are determined by E_a and E_d respectively. An unsurmountable antagonist is characterised by very slow offset of action or an irreversible action, and a slow onset of action.

β_2-receptor protein can vary in ways that have unexpected pharmacological consequences.

Selectivity of action in β-adrenoceptor agonists

We first encountered highly selective β_2-adrenoceptor agonism in 1966 when AH3021 and salbutamol were found to be very much more active on guinea-pig tracheal muscle than on heart muscle. Typical results for the saligenin series are given in Table 1 together with data showing similar but lesser selectivity of action in the corresponding catecholamines. Lands and his colleagues rationalised the catecholamine data by supposing that β-adrenoceptors exist in β_1 and β_2 subtypes (Lands *et al.*, 1967a,b). Our results provided strong support for this supposition and our conclusion was that β_1- and β_2-adrenoceptor proteins are chemically different and, since at that time we assumed that β-adrenoceptor agonists interact directly with adenylate cyclase, that the enzyme might exist in isoenzymic forms with minor differences in their amino acid sequences (Collin *et al.*, 1970). β_1- and β_2-adrenoceptor proteins were recently found to vary in much this way.

The cause of the highly selective action of salbutamol at β_2-receptors is obvious from the data in Table 1. The ratio of its potencies at β_2- and β_1-adrenoceptors is at least 500 whereas the corresponding ratio of its affinities for these receptors is only about 3. The main cause of its selective action is, therefore, its much greater efficacy at β_2-adrenoceptors. The same is probably true for all other highly selective non-catechol β_2-adrenoceptor agonists because the highest determined β_2/β_1 affinity ratio for any of them is only about 25 (Minneman *et al.*, 1979). There are not enough data to apportion the relative contributions of efficacy and affinity to the lesser β_2-selectivity of unnatural catecholamines.

Type 1 and Type 2 agonism

The link between salbutamol and salmeterol was the concept of two qualitatively different kinds of agonism which I called Type 1 and Type 2 (Jack, 1977, 1990).[2] In Type 1 agonism the affected cells are capable of responding continuously to continuing stimulation of physiological dimensions. In Type 2 agonism the cell, having responded to an effective stimulus, must recover before it can respond to another; the recovery process involves the regeneration by one means or another of sufficient free receptors to enable the agonist to elicit an effective stimulus.

The action of adrenaline at β-adrenoceptors is typical of Type 1 agonism because increasing the affinity of β-adrenoceptor agonists for their receptors, which prolongs the stimulus by increasing the duration of receptor occupancy, also increases their potency. Other examples of Type 1 agonism are the α-adrenoceptor effects of adrenaline and the actions of histamine, prostanoids, glucocorticoid steroids and many other physiological mediators and their analogues.

The archetype for Type 2 agonism is the action of acetylcholine at nicotinic cholinergic receptors. The paralysing effect of suxamethonium, the best known desensitising agonist, depends on its relatively persistent effect on acetylcholine receptors in the motor end-plates in skeletal muscle. There is no counterpart to suxamethonium in adrenergic pharmacology. However, the target cells in Type 2 agonism may also be inhibited by antagonists which, like Type 1 antagonists, have zero efficacy and high affinities for their receptors. +-Tubocurarine is an example of a competitive antagonist at cholinergic receptors in skeletal muscle.

Type 2 agonism may not be confined to very brief events such as neuromuscular transmission in skeletal muscle since the desensitising action of relatively stable potent LHRH analogues such as goserelin acetate on LH-secreting cells in the pituitary is similar in principle. Other LHRH analogues are competitive isosteric antagonists in this system (Jack, 1990; Lincoln, 1988).

I am far from sure that all cellular responses can be neatly classified as Type 1 or Type 2 events but still think that there is something in the idea. For example, it accounts for all the kinds of drug action achievable by isosteric actions of analogues of physiological mediators, namely, full, partial and desensitising agonism, and surmountable and unsurmountable antagonism. In any event, regardless of its ultimate merit, the concept has already proved useful by providing the hypothesis that led to salmeterol.

The discovery of salmeterol

Long before Fleming's mould and penicillin and certainly since then, chance has played a critical role in the discovery of new medicines. Salmeterol is yet another example because, quite apart from its effects being more beneficial than expected, it might never have been discovered had not a series of misfortunes delayed the marketing of salbutamol in the USA for nearly 10 years. The result was that it was already a mature drug with well known properties and established competition when it finally limped on to the American market in 1982. Something had to be done to create interest in it and so a closed symposium was held in Boston in 1981 at which leading physicians and scientists were asked to present their latest findings with the drug. Its proceedings were published and used to promote our products.

My contribution to the symposium was different from the others because I was asked to explore the possibility of a better β_2-adrenoceptor stimulant bronchodilator than salbutamol. By then, our β-adrenoceptor stimulant bronchodilator project had been abandoned after 15 years because of failure to separate the desirable inhibitory effects of salbutamol on bronchial muscle and mast cells from those which cause use-limiting side effects during its systemic administration. I had, therefore, to think about the problem for the first time in several years and

[2] In the original paper I did not make it clear that it is the cell and not the agonist which determines the kind of agonism. For example, the nicotinic effects of acetylcholine are Type 2 whereas its muscarinic effects on smooth muscle and secretory cells are Type 1.

concluded that there was still no reason for believing that the basic selectivity problem was solvable but that a better inhaled β_2-selective bronchodilator was feasible.

Ordinary inhaled doses of salbutamol induce near-maximal bronchodilation for 3 to 4 h without significant side-effects in nearly all patients and so its only serious defect seemed to be that its duration of action is too short to provide the 24 h cover needed to prevent nocturnal asthmatic attacks. Two ways of prolonging its action occurred to me. The first was that salbutamol, which is a base, is relatively slowly absorbed from the airways compared, for example, with cromoglycate, which is acidic, because its diffusion is impeded by reversible binding to acidic groups in mucoprotein. A drug which binds even more firmly to the mucoprotein would therefore be absorbed more slowly than salbutamol and, if sufficiently potent, would be longer acting (Jack, 1982).

The approach may be sound but it has never been tested because the second was simpler and seemed more likely to work. It was to make a β_2-adrenoceptor agonist which sticks firmly at its site of action in the receptor protein and remains efficacious for at least 8 h. The β-stimulant bronchodilator project was restarted after my return from Boston to test this idea.[3]

If the concept of Type 1 agonism was sound the required long-acting bronchodilator would bind exceptionally strongly at its receptors in the β_2-adrenoceptor protein and the corresponding $-\Delta G$, E_a and E_d values for the reaction would be unusually high (see Figures 2 and 3). The new drug would, therefore, be characterised by a slow onset of action (high E_a), a slower offset of action (high E_d), and high potency (high $-\Delta G$ for a given efficacy).

Such characteristics are most easily identified using superfused preparations of the kind described by Gaddum (1953) and changed by Vane (1982) into an exceptionally sensitive analytical method during his work on prostanoids and other mediators. We used superfused preparations of guinea-pig isolated tracheal muscle in which contraction was induced by prostaglandin $F_{2\alpha}$ in the superfusion fluid (Bradshaw *et al.*, 1987) or by electrical stimulation at 2 min intervals (Coleman & Nials, 1986). The test β-adrenoceptor agonists were added to the perfusion fluid.

In order to interpret the offset of action data it was necessary to have at least semi-quantitative estimates of rates of disengagement of agonists from their receptors. Accordingly, competitive binding studies using [^{125}I]-(−)-iodopindolol(^{125}IPIN), a specific high-affinity ligand for β-receptors, and selected β-adrenoceptor agonists were carried out by the method outlined in the legend to Figure 7.

A starting concern of some of the project team was the considerable literature on the desensitisation of β-adrenoceptors exposed to β-adrenoceptor agonists and

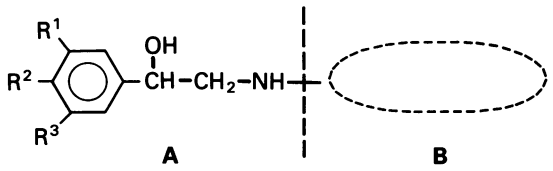


Figure 4 A hypothesis for a long-acting β_2-adrenoceptor agonist which forms a stable β_2-receptor protein complex.

the development of tolerance after chronic use of them by asthmatic patients (see, for example, Johnson & Fleming, 1989). If these reports were right, similar tolerance to the drug we sought was inevitable. My own view was that a waning response to β-adrenoceptor agonist bronchodilator treatment indicates worsening asthma and not desensitisation or down-regulation of β-receptors. It was strengthened at the Boston symposium when Dr Anne Tattersfield reported that no significant tolerance developed in asthmatic patients given as much as 500 μg salbutamol by inhalation four times daily for 4 weeks (Harvey & Tattersfield, 1982). The only way to settle the matter was to find and test the desired drug.[4]

The specific hypothesis that led to salmeterol is illustrated and explained in Figure 4; my favoured phenylethanolamine head was that of the clenbuterol series (Figure 5) because of its known high affinity for its receptors (Table 3). The required drug was, however, first sought in analogues of salbutamol with increasingly large non-polar phenylalkoxyalkyl *N*-substituents and eventually, by rational, painstaking optimisation, salmeterol emerged.

Pharmacological properties of salmeterol

Most of the experimental data in this section are taken from preliminary communications on the pharmacology of salmeterol (Ball *et al.*, 1987; Bradshaw *et al.*, 1987) or from a more comprehensive paper on its *in vitro* pharmacology which has been submitted to the British Journal of Pharmacology (Ball *et al.*, 1990). However, the interpretation of the data, like the starting hypothesis, is personal and does not necessarily represent the views of any of the other authors of these papers.

Salmeterol is similar to salbutamol in β_2-adrenoceptor selectivity and is about 10 times more active. The kinetics of its action are, however, quite different. As shown in Figure 6 its onset of action on tracheal muscle is slower than that of isoprenaline or salbutamol and it is much longer acting. Indeed, its main distinguishing characteristic is that its inhibitory effect on this preparation persists indefinitely even after superfusion with drug-free Krebs solution. Submaximal responses to the drug are similarly

[3] The project team was led by Dr Ian Skidmore under the overall direction of Dr Roy Brittain. Dr Larry Lunts, the originator of salbutamol, and Dr John Bradshaw were the senior chemists. The pharmacology reported in this paper was done by Dr Bob Coleman and his colleagues, and the receptor binding studies by Dr Mike Sumner and his colleagues. Dr Malcolm Johnson ultimately assumed responsibility for the definitive pharmacology of salmeterol and similar substances.

[4] The successful outcome of this project underlines the danger of too ready acceptance of *in vitro* or even *in vivo* data which preclude a positive hypothesis in drug research. I have great sympathy for Kenakin's view (1989) if he means that *in vitro* studies very often demonstrate what cells are capable of doing rather than what actually happens in the body.

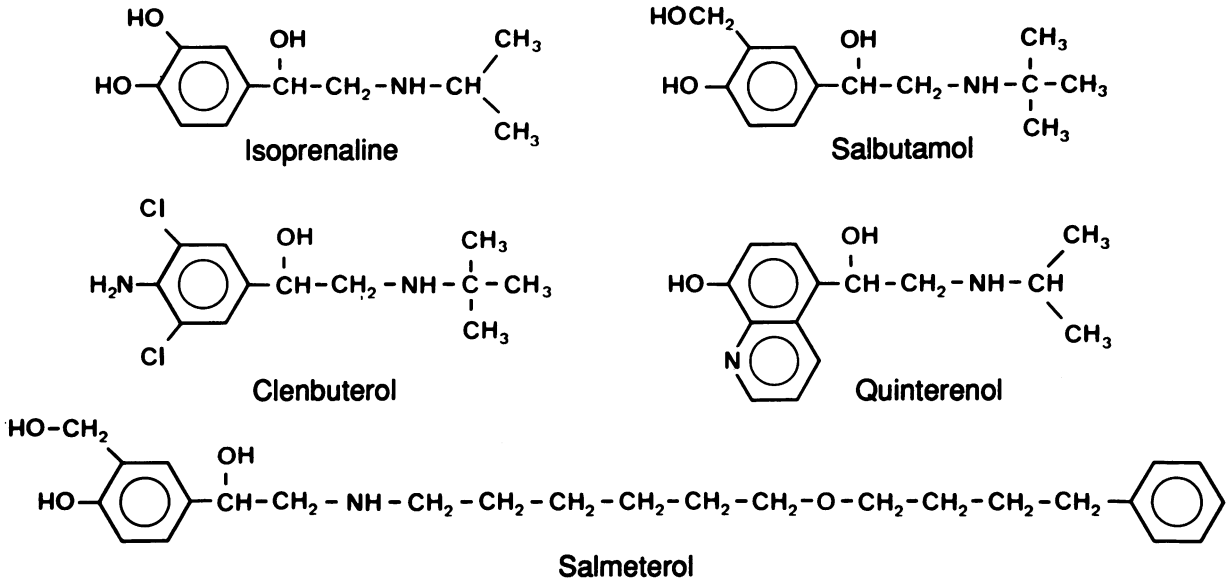


Figure 5 Chemical structures of β-adrenoceptor agonists selected for onset and offset of action studies.

Table 3 Characteristics of the actions of selected β-adrenoceptor stimulants on superfused guinea pig isolated respiratory muscle and on β_2-adrenoceptors derived from rat lung membrane

Agonist	*Relative equipotent concentration (EC_{50}) (−) isoprenaline = 1*	*Onset time (Ot_{50} min)*	*Recovery time (Rt_{50} min)*	n	*Rat lung β_2-adrenoceptors Relative affinity (−) isoprenaline = 1*	*Time for 50% dissociation (min)*
(−) Isoprenaline	1 (pEC_{50} ~ 7.7)	> 1 < 3	> 2 < 4	25	1 (pKi ~ 6.7)	≤ 4
(±) Salbutamol	3.0	3.2 (2.0–5.4)	11 (8–15)	8	10	≤ 4
(±) Clenbuterol	0.6	6.7 (4.5–8.9)	48 (39–57)	6	0.28	≤ 4
(±) Salmeterol	0.4	29 (24–36)	No recovery	14	0.07	≫ 150 in effect ∞
(±) Quinterenol	27	About 5	No recovery*	2	43	> 4 < 10
[^{125}I]-(−) Iodopindolol	–	–	–	–	–	Time for 50% association ~ 4

* Submaximal responses intensity during drug washout.

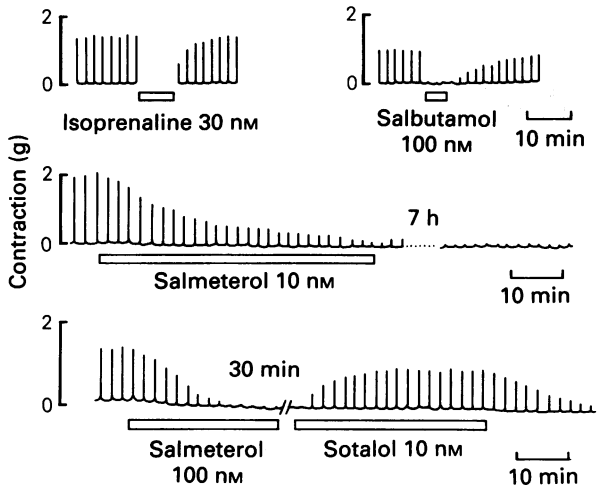


Figure 6 Effects of isoprenaline, salbutamol and salmeterol on electrically stimulated superfused guinea pig isolated trachea and of sotalol on the response to salmeterol.

persistent and constant. Another unusual feature of its action that requires explanation is its ready competitive reversal by sotalol and similar β-adrenoceptor blockers, and its reassertion when superfusion with the β-adrenoceptor blocker is discontinued.

Salmeterol has similarly persistent inhibitory actions on the release of histamine and other mediators of inflammation from antigen-challenged IgE-sensitised rat mast cells and fragments of human lung, actions which are also reversed by β-adrenoceptor blockade (Butchers *et al.*, 1987).

The persistent selective β_2-adrenoceptor stimulant effect of salmeterol is also evident in whole animals and man. For example, the inhaled drug protects guinea pigs from the bronchoconstricting action of histamine for much longer than salbutamol. It is similarly longer acting than salbutamol in asthmatic patients in whom 50 or 100 μg inhaled doses induce bronchodilatation which is maximal after about 1 to 2 h and is still very obvious

after 12 h and more (Ullman & Svedmyer, 1988). β_2-adrenoceptor mediated side-effects such as tremor and tachycardia are few in patients given the drug by inhalation but are obvious and persistent after systemic administration.

Another very significant property of salmeterol is its inhibitory action on the early and late responses and other sequelae to allergen challenge in asthmatic patients (Twentyman *et al.*, 1990). The therapeutic significance of this and related results is considered later. First, however, we must establish why salmeterol is so long-acting and why its β_2-adrenoceptor stimulant action is antagonised during β-adrenoceptor blockade.

The mechanism of action of salmeterol

The results of an experiment critical to understanding the mechanism of action of salmeterol are illustrated in Figure 7. ^{125}IPIN was rapidly taken up by β_2-adrenoceptors preloaded with isoprenaline or salbutamol when the system was diluted. Accordingly both drugs readily dissociate from their receptors. In stark contrast, the β-specific ligand was not taken up by the salmeterol-loaded receptors. This is strong, if indirect, evidence

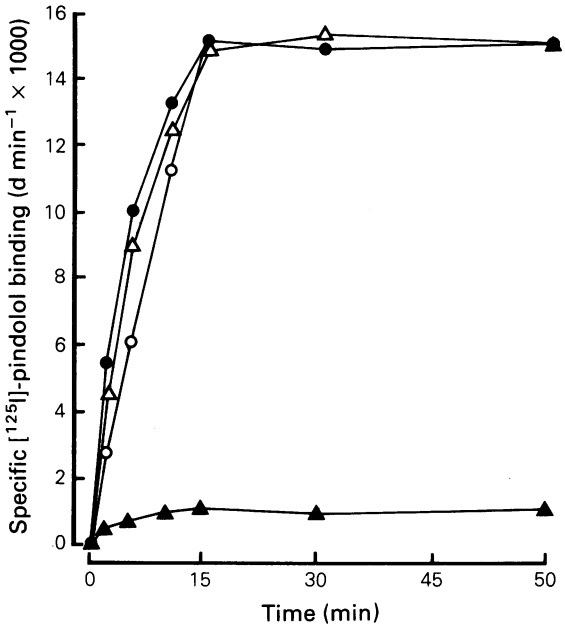


Figure 7 Time courses for the specific binding of [^{125}I]-(−)-iodopindolol to β-adrenoceptors in fragments of rat lung membranes pretreated with vehicle (●), 100 μM (−)-isoprenaline (○), 300 μM salbutamol (△) or 10 μM salmeterol (▲). Membranes were treated for 30 min at 37° C with sufficient drug to occupy > 90% of receptors. Drug dissociation was then initiated by 300 fold dilution with buffer containing [^{125}I]-(−) iodopindolol (100 pM) and the time course for radioligand binding to each membrane preparation was determined. The data are from a single representative experiment and were reproduced at least three times.

that salmeterol does not dissociate from its receptors under these conditions (Brittain *et al.*, 1988).

This result presents what appears to be a dilemma. Why is uptake of β-adrenoceptor-specific ^{125}IPIN inhibited but not that of pindolol or other β-adrenoceptor blockers which reverse the action of salmeterol? The probable explanation is illustrated in Figure 8 (see p. 509). ^{125}IPIN, by virtue of its 2-iodosubstituent, is a bigger molecule than pindolol or propranolol and this is enough to deny it access to the physiological β_2-adrenoceptor when salmeterol is in place.

We can now explain why the pharmacological effects of salmeterol on tracheal muscle are different from those of isoprenaline. Isoprenaline, as shown in Figure 11, associates with and dissociates from its receptors quickly and so its onset and offset of action are relatively fast; R^{Iso} is assumed to revert quickly to the ground state R^{o}. By contrast, salmeterol, a much larger molecule, associates with its receptors slowly and never dissociates from them. However, the active complex, $SmR^{Sm(a)}$, alternates with the inactive complex, $SmR^{Sm(b)}$ (Figure 11). In the former conformation, the physiological β_2-adrenoceptor is occupied by the phenylethanolamine head and the resultant complex is catalytically active. In the latter, that receptor is free to react with β-adrenoceptor blockers such as sotalol which derange and inhibit the receptor enzyme. When the β-adrenoceptor blocker dissociates, the agonist is still in place and β_2-adrenoceptor agonism resumes. For this reason, I consider salmeterol to be a captive agonist which is part of an unnatural, self-activating ground-state receptor protein, $SmR^{Sm(b)}$.

If β-adrenoceptor blockers can bind to their receptors in $SmR^{Sm(b)}$ and thereby antagonise the β_2-adrenoceptor effect of salmeterol, so may β-adrenoceptor agonists bind to their receptors in that protein to form active complexes. Furthermore, β-adrenoceptor agonists of greater efficacy will augment the salmeterol stimulus while they are in position. The basic salmeterol action resumes when the other agonist is washed out.[5]

If we have correctly deduced how salmeterol acts and interacts with agonists and antagonists the following questions arise. What determines the potency of a captive β_2-adrenoceptor agonist? What determines its duration of action?

Potency and duration of action of captive β_2-adrenoceptor agonists

The potency of a captive β_2-adrenoceptor agonist (Ag) is determined by the proportion of time its receptor protein complex exists in its active conformation, $AgR^{Ag(a)}$, and by the efficacy of that complex. The former is determined by the decrease in free energy for the reaction $AgR^{Ag(b)} \rightarrow AgR^{Ag(a)}$ which may be measured by competitive binding studies using a tritium-

[5] The concurrent use of a second β-adrenoceptor agonist with salmeterol is unnecessary and undesirable in patients. That drug on its own provides an adequate prolonged stimulus which, being self-limiting, is most unlikely to induce tolerance. If salmeterol, used correctly, gives inadequate control in asthma, appropriate steroid treatment is indicated. β-adrenoceptors are contraindicated during salmeterol treatment because, apart from their well-known bronchoconstricting action in asthmatic patients, they reverse the unique anti-inflammatory action of the drug.

labelled specific ligand, of known affinity for the β_2-adrenoceptor, which can gain access to it. In general, agonists whose phenylethanolamine heads have high affinities for the region of the physiological receptor are more active for particular efficacies. This is why analogues of salmeterol containing the high affinity phenylethanolamine groups present in clenbuterol or soterenol are more potent than their saligenin counterparts (UK Patents 2165542A, and 2162842A and 993584 respectively).

Since salmeterol does not dissociate from its receptors and continues to be efficacious, the slow waning of its bronchodilating action that occurs in patients after 10–12 h is probably attributable to internalisation and catabolism of occupied receptors and their replacement with new receptors. The latter are, of course, occupied by the next dose of the drug and so twice daily dosage provides 24 h cover. A corollary to this explanation is that, whereas more potent captive agonists than salmeterol are already known, longer acting drugs are less likely since this requires them to reduce the rate of turnover of the β_2-adrenoceptor protein in the lungs.

The binding of salmeterol and other unnatural β-adrenoceptor agonists at the β-adrenoceptor protein

The elongated structure for salmeterol depicted in Figure 5 is intended only to emphasise the fact that it is a much larger molecule than isoprenaline or salbutamol. Its large non-polar *N*-substituent also makes it much more lipophilic and less soluble in water. The reader should, however, be aware that the fully extended structure depicted does not exist in nature because the salmeterol molecule is extremely flexible and adapts its conformation in whatever environment to form a system of minimum free energy. Thus, so far as they are able to, its polar phenylethanolamine grouping associates with polar groupings, and its non-polar *N*-substituent with non-polar groups in each environment. Accordingly, binding of salmeterol at its receptors in the β_2-adrenoceptor protein involves interactions between its phenylethanolamine head and the area of the protein which normally binds adrenaline, and between its *N*-substituent and readily accessible non-polar groups in the receptor protein. The ether oxygen in the *N*-substituent must play a critical guiding role in the latter process because it is essential for optimum activity.[6] Where does the *N*-substituent bind?

At this point I turned for help to my friends in ICI Pharmaceuticals because of their unique experience of interactions between adrenaline analogues and β-adrenoceptor proteins. The outcome, thanks to Dr Tom McKillop, was an introduction to Dr Dave Timms and his colleagues in the ICI computer modelling department. My further good luck was that they already had 'in stock' a computer model of bacteriorhodopsin which is the archetype for all receptor proteins which process G-proteins; it was the first to be characterised and is still the only one whose tertiary structure has been experimentally determined (Henderson & Unwin, 1975). Timms' computer model for bacteriorhodopsin is shown in Figure 9 in which the arrangement of its seven hydrophobic transmembrane α-helices is clear.

Using this information and that generated by Dixon and his colleagues in Merck, Sharp and Dohme, USA (Strader *et al.*, 1989) which indicates that the binding of adrenaline at its receptors involves an ionic interaction between its basic centre and an aspartate carboxylate group in α-helix III of the β_2-receptor protein and hydrogen bonding between its catechol group and serine residues 204 and 207 in α-helix V, Timms concluded that, in its active complex, $SmR^{Sm(a)}$, the phenylethanolamine head of salmeterol is located similarly to adrenaline in the receptor protein, and that its non-polar *N*-substituent is accommodated within the hydrophobic core of the protein. His model for $SmR^{Sm(a)}$ is shown in Figure 10, together with an illustration of the binding of adrenaline. It is noteworthy that the terminal phenylbutoxy group of salmeterol is anchored in a hydrophobic pocket in the core and that it is the number and arrangement of the hydrophobic interactions involved in the binding of the *N*-substituent which fixes it irreversibly within the receptor protein.

Timms' model also brings reality to the diagrammatic representation of the pharmacological receptors for the simpler adrenaline analogues in Figure 3. The binding of their *N*-substituents also occurs in the hydrophobic core of the receptor protein.

The therapeutic significance of salmeterol

The Allen and Hanbury's/Glaxo approach to asthma through the years has been to use a β_2-adrenoceptor agonist by inhalation to dilate the bronchi and to supplement it as required by an inhaled and, if need be, a systemic anti-inflammatory steroid to contain and reverse the life-threatening mucosal damage and hypersecretion which characterises worsening disease. Very simply, our purpose is to keep the airways open and 'clean'. I was, therefore, first incredulous and then delighted as the results of pharmacological and clinical studies in patients indicated with increasing certainty that salmeterol, used alone, possesses both bronchodilating and anti-inflammatory activities.

Salmeterol was recently marketed in the United Kingdom and so full accounts of its clinical trials will be published soon. All that need be said now is that 50 μg (or 100 μg in difficult cases) of the drug, given by inhalation night and morning, was obviously more effective than inhaled salbutamol, 200 μg four times daily, in controlled studies of many months duration in chronic asthmatics. The virtual abolition of nocturnal asthma during salmeterol treatment was particularly pleasing since this was the starting objective of the project.

Equally satisfying were the absence of tolerance even

[6] The binding of salmeterol at its receptors is accompanied not only by a substantial decrease in free energy but by increased entropy, since binding of its *N*-substituent involves replacement of lipid-water interfaces with lipid-lipid interfaces. These energy changes are, of course, accompanied by other free energy and entropy changes caused by conformational changes in the receptor protein as it accommodates the agonist.

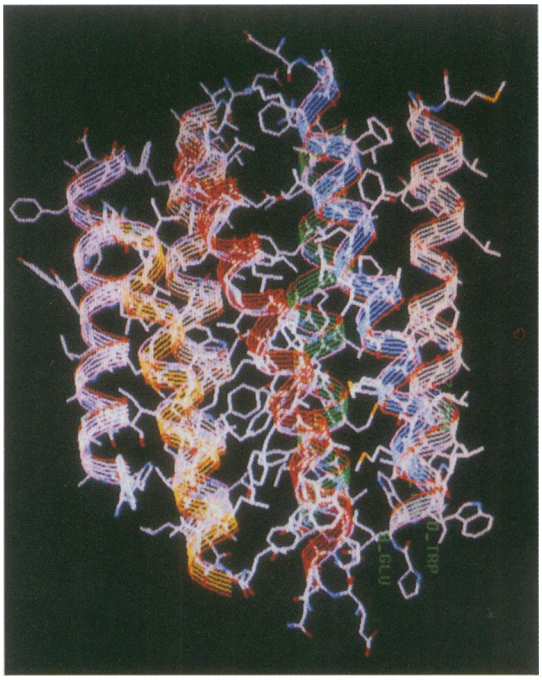


Figure 9 (a)

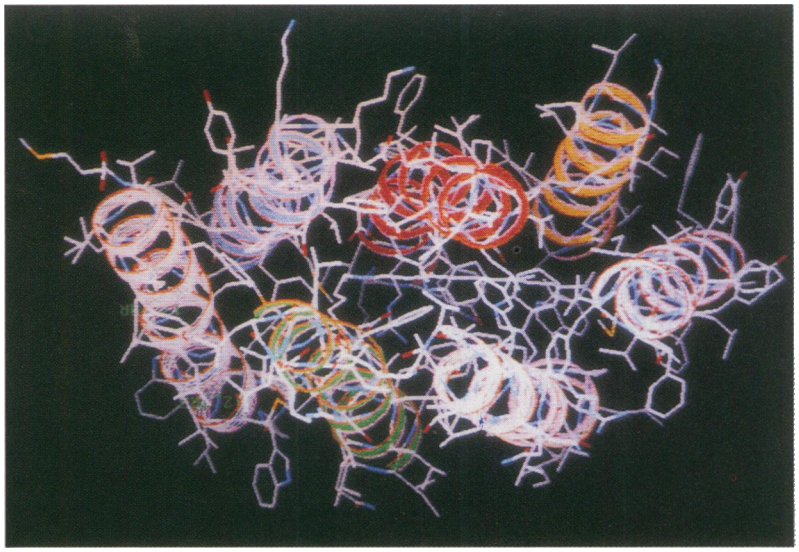


Figure 9 (b)

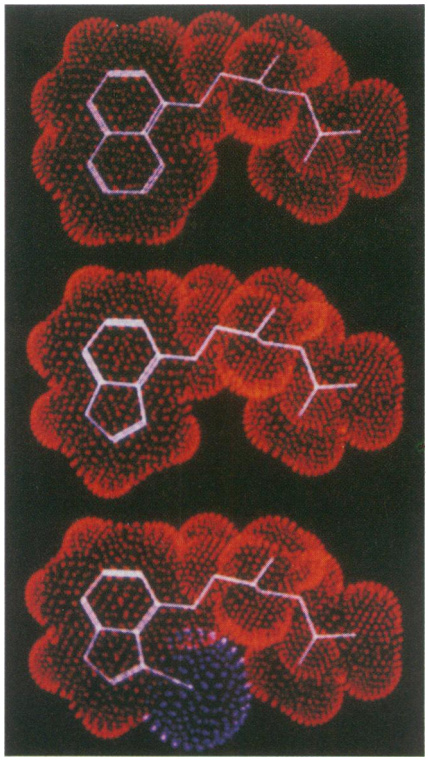


Figure 8

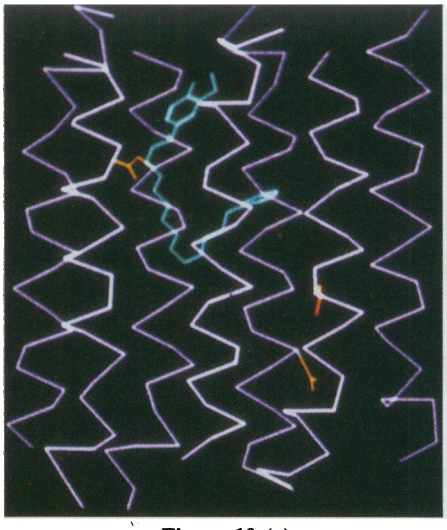


Figure 10 (a)

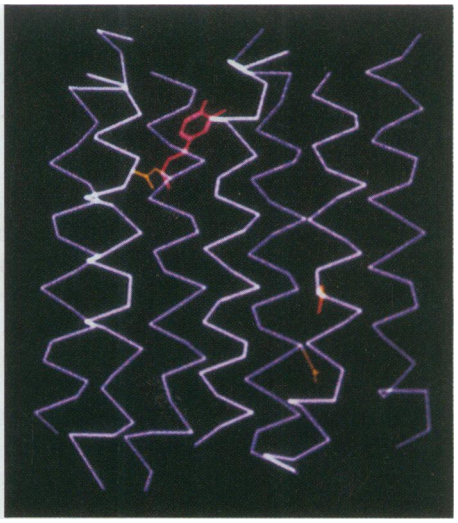


Figure 10 (b)

Figure 8 Computer models of propranolol (top), pindolol (middle) and 2-iodopindolol (bottom) illustrating the steric effect of the 2-iodosubstituent by means of van der Waals radii. (Courtesy of ICI Pharmaceuticals.)

Figure 9 Computer model of bacteriorhodopsin a) trans-membrane view, b) view of external surface (Courtesy of ICI Pharmaceuticals).

Figure 10 Computer skeleton models of bacteriorhodopsin showing probable binding sites for adrenaline in red a) and salmeterol in green b) in the analogous β_2-receptor protein. (Courtesy of ICI Pharmaceuticals.)

after many months of treatment and the low incidence of use-limiting side-effects (Palmer, 1990, personal communication). We have already established why salmeterol is such a long-acting bronchodilator. Let us now examine the evidence for a clinically important anti-inflammatory action and its possible cause.

The first indication that salmeterol is more than an exceptionally long-acting bronchodilator was a clinical observation that patients treated for months with standard inhaled doses of the drug deteriorated much more slowly than controls treated with salbutamol when treatment was stopped. Salbutamol-treated patients needed to use their inhalers within a few hours whereas equivalent deterioration took at least 4 days to develop after salmeterol treatment. That the difference was real and attributable to an anti-inflammatory action is consistent with the bronchoscopic finding in a few patients that the bronchi during prolonged salmeterol treatment are clean compared with the typically inflamed airways during salbutamol treatment (Brittain & Palmer, 1990, personal communication). The latter condition is consistent with the recent demonstration in New Zealand that regular treatment with inhaled fenoterol, 200 μg four times daily, for 24 weeks provided poorer control of asthma than the same drug or salbutamol or terbutaline used by inhalation as required by the patients (Sears *et al.*, 1990). Accordingly, with the possible exception of formoterol, which has not yet been adequately tested, salmeterol may be the only β_2-adrenoceptor agonist indicated for regular maintenance treatment of asthma. It is also almost certainly superior to the others for intermittent use.

A second indication of a specific anti-inflammatory action was that a single 50 μg inhaled dose of salmeterol protected asthmatic volunteers from all the adverse effects of allergen challenge for at least 34 h. These comprise the early and late asthmatic responses and bronchial hyperreactivity to 'non-specific' stimuli (Twentyman *et al.*, 1990). Until recently, this result would have been regarded as strong evidence of an important qualitative difference between salmeterol and salbutamol because the latter was believed to inhibit only the early response to allergen challenge. However, this distinction may not be absolute in view of two recent reports that salbutamol and formoterol are also capable of preventing the late response (Palmqvist *et al.*, 1989; Twentyman *et al.*, 1990).

The anti-inflammatory action of salmeterol probably depends on its ability to induce long-lasting inhibition of the release of pro-inflammatory mediators from mast cells (Butchers *et al.*, 1987) and alveolar macrophages (Baker & Fuller, 1990), and to stabilise the respiratory mucosa when it is exposed to toxic agents such as histamine as evidenced by its preventing leakage of plasma proteins into the airways (Johnson, 1990). The first and last of these actions are almost certainly of β_2-adrenergic origin since they are reversible by β-adrenoceptor blockade in essentially the same way as the inhibitory effect of the drug on tracheo-bronchial muscle. The evidence for β-adrenergic inhibition of macrophage function is, however, conflicting but, following William of Occam, I am reluctant to invoke another mechanism until it is essential to do so.

Evidence in favour of a β-adrenergic mechanism is contained in a recent excellent paper from Sweden which shows that human alveolar macrophages bear functioning β-adrenoceptors whose activation by adrenaline or isoprenaline causes increased intracellular levels of cyclic AMP (Hjemdahl *et al.*, 1990). This action of isoprenaline is inhibited by (−)-propranolol (pD_2 8.2) and, because the β_2-selective ICI 118551 (pD_2 7.5) is relatively more active than the β_1-selective atenolol (pD_2 5.6), the authors concluded that there is probably a preponderance of β_2-adrenoceptors in the macrophages. Unfortunately they did not test salbutamol or salmeterol in their system nor did they determine the effect of isoprenaline on the release of mediators from IgE-sensitised macrophages after allergen challenge. Evidence against a β-adrenergic mechanism of a known kind is offered by an equally competent British group who found that salmeterol, but not salbutamol, inhibits the release of mediators from such macrophages and that its effect is not inhibited by propranolol (Baker & Fuller, 1990).

The mechanism of the anti-inflammatory action of salmeterol will undoubtedly be clarified by experiment in the near future. If it is not a β-adrenergic action, medicinal chemists will have a splendid opportunity to make even better anti-inflammatory agents to treat asthma and possibly other diseases. In the meantime, asthmatic patients may take comfort in the fact that salmeterol is a much better medicine than earlier β-adrenoceptor agonists.

More interactions between unnatural β-adrenoceptor agonists and the β_2-adrenoceptor protein

During the salmeterol project, the rates of onset and offset of action of a variety of β-adrenoceptor agonists were determined using superfused guinea-pig isolated tracheal preparations in order to evaluate the starting hypothesis. These experiments were supplemented by receptor binding studies using ^{125}IPIN as outlined in Figure 7 to assess their affinities for, and their rates of dissociation from their receptors in the β_2-adrenoceptor protein. The structures of some of the agonists tested are given in Figure 5. The purpose of this section is to describe and explain some of the remarkable results obtained.

Isoprenaline was tested because it is the archetypal non-selective, specific β-adrenoceptor agonist and salbutamol as its highly β_2-selective counterpart. Their affinities for their receptors were known to be quite low and so their onsets and offsets of action were expected to be relatively fast. Clenbuterol was chosen because of its known high affinity for its receptors and its low efficacy; both its onset and offset of action were expected to be significantly slower than those of isoprenaline. Quinterenol was tested because its remarkable properties had troubled me since the drug was first described by Scriabine and his colleagues in 1968. I wrote the following about it in 1970. 'Clearly quinprenaline (quinterenol) is an unusual β-adrenoceptor stimulant in being selective in its *in vitro* actions and virtually non-selective *in vivo*. Other unusual features are its slow onset and very long duration of action, and its high affinity for β-adrenoceptors as shown by the difficulty in terminating drug

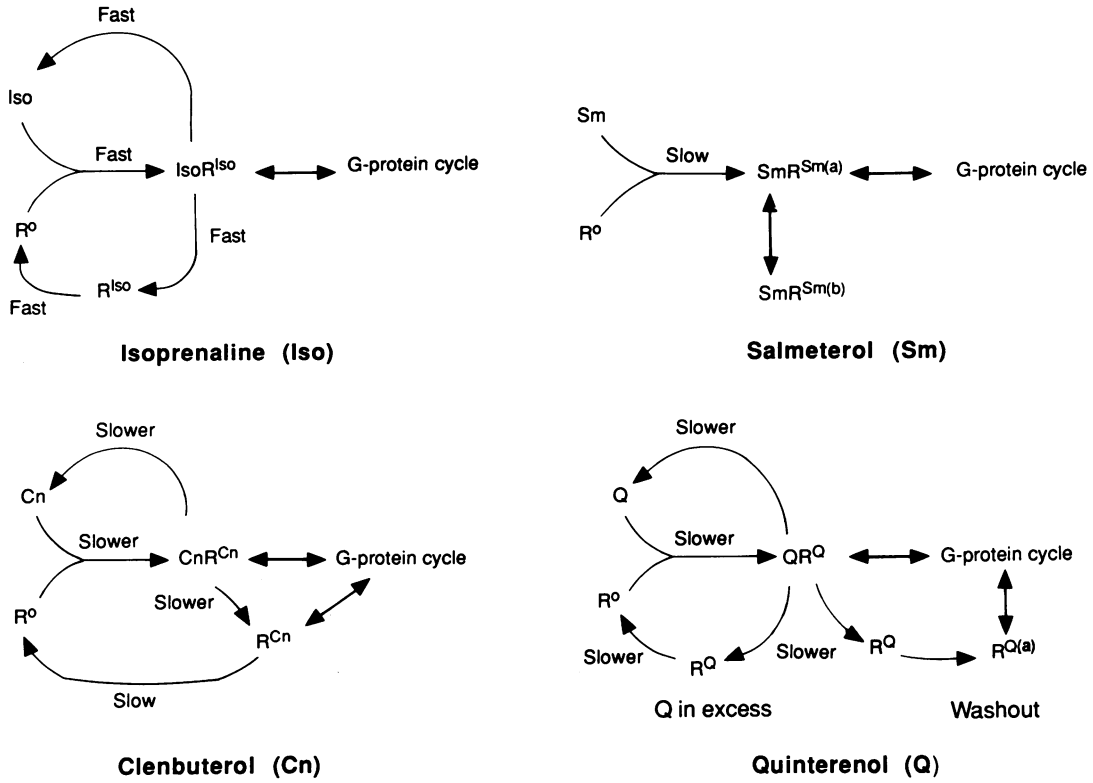


Figure 11 Diagrams illustrating four different kinds of interactions between β-adrenoceptor agonists and the β_2-receptor protein (R^o). In $SmR^{Sm(a)}$, the region of the physiological β_2-receptor is occupied by the phenylethanolamine head of salmeterol. In $SmR^{Sm(b)}$ it is unoccupied. $R^{Q(a)}$ is a permanently activated form of the receptor protein.

action simply by washing the tissues' (Brittain *et al.*, 1970). These properties are so like those of salmeterol that my supposition that quinterenol persists at its receptors still seemed reasonable 15 years later. There was, however, still no obvious physico-chemical basis for such behaviour.

The results of these experiments and the affinities of the chosen β-adrenoceptor agonists for their receptors in rat lung membrane are summarised in Table 3. In the interpretation which follows, the reference compound is isoprenaline.

The kinetics of the actions of isoprenaline and salmeterol have already been compared and explained and how they interact with the β_2-adrenoceptor protein is illustrated in Figure 11.

The slightly slower onset of action of salbutamol compared with isoprenaline was to be expected because of its lesser efficacy, but its clearly slower offset of action was not, particularly since the rates of dissociation of these drugs from their receptors were found to be similar. One possible cause of the longer action of salbutamol is that it, unlike isoprenaline, is not a substrate for cellular uptake processes for catecholamines and would, therefore, persist for longer at its site of action during the wash-out process. However, another more likely explanation became obvious when the clenbuterol results were obtained.

The relatively slow onset and offset of action of clenbuterol were exactly what had been anticipated for a β-adrenoceptor agonist of low efficacy and high affinity for its receptors. I was, therefore, greatly surprised when its rate of dissociation from its receptors was found to be similar to that of isoprenaline and obviously too fast to be the cause of its slow offset of action. In my view, therefore, its prolonged action, unlike that of salmeterol, does not result from its persisting at its site of action but from the receptor protein continuing to be catalytically active after the agonist has dissociated from its receptor protein complex.

The interactions envisaged between clenbuterol and the β_2-adrenoceptor protein are illustrated in Figure 11. The tertiary structure of the catalytically active, unnatural, drug-free β_2-adrenoceptor protein, R^{Cn}, is different from and more stable than the corresponding structure, R^{Iso}, induced by isoprenaline, and the biological response is more persistent because it reverts more slowly to the ground state, R^o. The delayed offset of action of salbutamol may have a similar cause. If R^{Sal} were intermediate in stability between R^{Iso} and R^{Cn} its offset of action would be slower than that of isoprenaline but faster than that of clenbuterol.

I believe this to be the first report of the maintenance of drug action by an agonist-free activated receptor protein.

I have dealt with quinterenol last because it is undoubtedly the most intriguing β-adrenoceptor agonist I have ever encountered. Bob Coleman and his colleagues (Nials *et al.*, 1990) have confirmed Scriabine's finding that the onset of its β_2-adrenoceptor action is slow, and its effect persistent. However, they also found two very significant differences between its action and that of salmeterol. The first is that submaximal responses to quinterenol intensify and become permanent when the tracheal preparation is superfused with drug-free Krebs solution to remove the drug, whereas those to salmeterol remain constant. The second is that quinterenol dissoci-

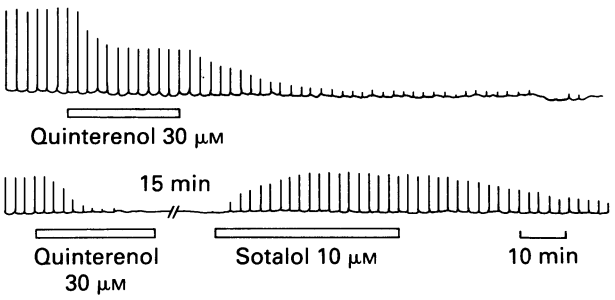


Figure 12 Action of quinterenol on electrically stimulated superfused guinea pig isolated trachea (upper trace) and its temporary reversal by sotalol (lower trace).

ates fairly readily from its receptors whereas salmeterol is permanently bound to the β_2-adrenoceptor protein. These properties of quinterenol, which are summarised in Table 3 and illustrated in Figure 12, lead inevitably to the remarkable conclusion that its permanent inhibitory action results from the formation, during washout of the drug, of a stable unnatural drug-free conformation of the β_2-adrenoceptor protein which is permanently activated or self-activating.

The interactions postulated between quinterenol and the β_2-adrenoceptor protein in Figure 11 are compatible with its pharmacological properties and are thermodynamically feasible. When, as on the left of the model, quinterenol is present in excess and the ratio of drug to receptor protein molecules is substantially greater than 1, the drug behaves like any other modestly active β_2-adrenoceptor agonist. For example, a dose-response curve is obtainable simply by progressively increasing its concentration in the perfusion fluid. The unusual features of its actions occur when the ratio of drug to receptor protein molecules falls below 1 during the wash-out process because the receptor protein adopts a new stable activated conformation, $R^{Q(a)}$, which is more efficacious than QR^Q or R^Q. The overall effect of quinterenol is, therefore, to catalyse the conversion of the ground state receptor protein, R^o, into a stable active conformation, $R^{Q(a)}$.

One obvious question remains. Why is $R^{Q(a)}$ not formed when quinterenol is present in excess? A possible answer is that the active quinterenol-receptor protein complex, depicted as QR^Q in Figure 11, is not simply a 1:1 complex but contains more than one drug molecule, only one of which is bound in the region of the physiological receptor and the other(s) elsewhere in the protein. R^Q contains only the latter stabilising molecules of drug and, with drug in excess, it may oscillate between active and inactive conformations or recombine with Q to form QR^Q. As the drug is washed out, the thermodynamically favoured sequence of changes is different, namely, $QR^Q \xrightarrow{-Q} R^Q \xrightarrow{-Q} R^{Q(a)}$ which is the permanently activated conformation of the β-adrenoceptor protein.

Summing up

A distinguishing feature of good research is that a successful outcome is always attended by significant theoretical and/or practical consequences. The salmeterol project had both.

The immediate practical consequence was salmeterol itself, the first anti-inflammatory β-adrenoceptor bronchodilator. I am sure that its impact on the treatment of asthma will be at least as great as that of salbutamol or beclomethasone dipropionate, each of which was similarly novel in its time. The transformation of the treatment of the disease achieved with these drugs is the greatest contribution to therapeutics by the Ware research group so far.[7]

When using salmeterol it is essential to remember that its unique persistent bronchodilating action and most, if not all, of its anti-inflammatory action in chronic asthma result from a permanent increase in cyclic AMP to efficacious but not excessive levels in bronchial muscle and other cells in the lungs. This condition is achieved by regular twice daily inhalations of salmeterol, because it fixes irreversibly to the β_2-receptor protein and activates it repeatedly, but this does not happen with shorter acting β_2-adrenoceptor stimulants such as salbutamol. It is also important to remember that the effectiveness of salmeterol in asthmatic patients may be greatly diminished if for any reason the critical cyclic AMP levels are allowed to fall because activation of inflammatory mechanisms may then be induced by allergens, including those already fixed to inhibited pro-inflammatory cells. The drug must, therefore, be taken regularly in chronic asthma and concurrent administration of β-adrenoceptor blocking drugs is contraindicated.

The theoretical significance of this work arises from the findings that, contrary to prevailing expert opinion, continuous controlled β_2-adrenoceptor agonism did not induce pharmacological tolerance in relatively short-term *in vitro* preparations or in patients treated for long periods, and that isoprenaline, salbutamol or clenbuterol, salmeterol and quinterenol interact with the β_2-adrenoceptor protein in distinctly different ways which are reflected in their pharmacological properties. I am sure that these are only particular examples of a wide range of pharmacological possibilities because the functional relationship between adrenaline and the β_2-adrenoceptor protein is similar to that between a great variety of physiological mediators and their receptor proteins, nearly all of which exist in iso-receptor forms. It is, therefore, very likely that each of these systems, and others which do not involve G-proteins, can be manipulated in a similar way to yield new kinds of selectively acting drugs of varying durations of action and different therapeutic uses. Drug researchers will not be short of worthwhile projects for a very long time.

I am greatly indebted to all my former colleagues in Allen and Hanburys and Glaxo whose efforts gained me the Lilly award and remember especially the colleagues and friends who laid the foundations of the large research institution now at Ware. It is a great pity that Alec Ritchie and Ivor Mitchell did not live to see this consequence of their efforts.

I offer grateful thanks to Tom McKillop, Dave Timms and

[7] This achievement will soon be equalled or surpassed by the work of Patrick Humphrey and his colleagues on selectively acting 5-hydroxytryptamine analogues. The project, which was started in 1972, has already yielded two new kinds of clinically proven drugs, ondansetron which is, *inter alia*, a much superior antiemetic, and sumatriptan, the first truly specific treatment for migraine. More will follow.

their colleagues in ICI Pharmaceuticals for their kindness and help with the computer modelling.

I am especially grateful to my wife, Lydia, for unfailing encouragement and support during the difficult periods that inevitably accompany a career in research and development, and for the many more important things in our lives.

References

Baker, A. J. & Fuller, R. W. (1990). Anti-inflammatory effect of salmeterol on human alveolar macrophages. *Am. Rev. resp. Dis.*, **141**, A394.

Ball, D. I., Brittain, R. T., Coleman, R. A., Denyer, L. H., Jack, D., Johnson, M., Lunts, L. H. C., Nials, A. T., Sheldrick, K. E. & Skidmore, I. F. (1990). Salmeterol, a novel long-acting β_2-adrenoceptor agonist: characterisation of pharmacological activity *in vitro*. *Br. J. Pharmac.* (in press).

Ball, D. I., Coleman, R. A., Denyer, L. H., Nials, A. T. & Sheldrick, K. E. (1987). *In vitro* characterisation of the β_2-adrenoceptor agonist, salmeterol. *Br. J. Pharmac.*, **92**, 591P.

Bilezikian, J. P., Dornfeld, A. M. & Gammon, D. E. (1978). Structure-binding-activity analysis of beta-adrenergic amines—1. Binding to the beta receptor and activation of adenylate cyclase. *Biochem. Pharmac.*, **27**, 1445–1454.

Birnbaumer, L., Yatani, A., Vandongen, A. M. J., Graf, R., Codina, J., Okabe, K., Mattera, R. & Brown, A. M. (1990). G-protein coupling of receptors to ionic channels and other effector systems. *Br. J. clin. Pharmac.*, **30**, 13S–22S.

Bradshaw, J., Brittain, R. T., Coleman, R. A., Jack, D., Kennedy, I., Lunts, L. H. C. & Skidmore, I. F. (1987). The design of salmeterol, a long-acting selective β_2-adrenoceptor agonist. *Br. J. Pharmac.*, **92**, 590P.

Brittain, R. T., Dean, C. M. & Jack, D. (1976). Sympathomimetic bronchodilator drugs. *Pharmacology and Therapeutics Bulletin*, **2**, 423–462.

Brittain, R. T., Jack, D. & Ritchie, A. C. (1970). Recent β-adrenoceptor stimulants. In *Advances in Drug Research*, **5**, pp 197–253, eds Harper, N. J. & Simmonds, A. B. London: Academic Press.

Brittain, R. T., Jack, D. & Sumner, M. J. (1988). Further studies on the long duration of action of salmeterol, a new, selective beta$_2$-stimulant bronchodilator. *J. Pharm. Pharmac.*, **40**, 93P.

Butchers, P. R., Cousins, S. A. & Vardey, C. J. (1987). Salmeterol: a potent and long-acting inhibitor of the release of inflammatory and spasmogenic mediators from human lung. *Br. J. Pharmac.*, **92**, 745P.

Coleman, R. A. & Farmer, J. B. (1971). The inducement of tone and its inhibition in isolated tracheal muscle. *J. Pharm. Pharmac.*, **23**, 220–223.

Coleman, R. A. & Nials, A. T. (1986). The characterisation and use of the electrically stimulated, superfused guinea-pig tracheal strip preparation. *Br. J. Pharmac.*, **91**, 409P.

Coleman, R. A., Nials, A. T., Sheldrick, K. E. & Sheldrick, R. L. G. (1986). A novel and versatile multiple superfusion system: a replacement for the organ bath? *Br. J. Pharmac.*, **91**, 408P.

Collin, D. T., Hartley, D., Jack, D., Lunts, L. H. C., Press, J. C., Ritchie, A. C. & Toon, P. (1970). Saligenin analogues of sympathomimetic catecholamines. *J. med. Chem.*, **13**, 674–680.

Gaddum, J. H. (1953). The technique of superfusion. *Br. J. Pharmac.*, **8**, 321–326.

Gilman, A. G. (1987). G-proteins: transducers of receptor-generated signals. *Ann. Rev. Biochem.*, **56**, 615–649.

Harvey, J. E. & Tattersfield, A. E. (1982). Airway response to salbutamol: effect of regular salbutamol inhalations in normal, atopic and asthmatic subjects. *Thorax*, **37**, 280–287.

Henderson, R. & Unwin, P. N. T. (1975). Three dimensional model of purple membrane obtained by electron microscopy. *Nature*, **257**, 28–32.

Hjemdahl, P., Larsson, K., Johansson, M., Zetterlund, A. & Eklund, A. (1990). β-adrenoceptors in human alveolar macrophages isolated by elutriation. *Br. J. clin. Pharmac.*, **30**, 673–682.

Jack, D. (1977). Rational approaches to drug discovery. In *Advanced Medicine – Topics in Therapeutics*, ed. Shanks, R. G., **3**, 135–147, London: Pitman Medical.

Jack, D. (1982). Albuterol: are improved selective beta$_2$-stimulant bronchodilators attainable? (1982). In *New Concepts in the Topical Treatment of Asthma and Related Disorders*, pp. 23–27. Proceedings of a symposium sponsored by Brigham and Womens Hospital and Harvard Medical School. Cliggott Publishing Co.

Jack, D. (1990). Safety by design. *Drug Safety*, **5** (suppl.), 4–23.

Johnson, M. (1990). The pharmacology of salmeterol. *Lung*, **168** (suppl.), 115–119.

Johnson, S. M. & Fleming, W. W. (1989). Mechanisms of cellular adaptive sensitivity changes; applications to opioid tolerance and dependence. *Pharmac. Rev.*, **41**, 435–488.

Kenakin, T. P. (1989). Challenges for receptor theory as a tool for drug and drug receptor classification. *Trends Pharmac. Sci.*, **10**, 18–22.

Lands, A. M., Arnold, A., McAuliff, J. P., Luduena, F. P. & Brown, T. G. (1967a). Differentiation of receptor systems activated by sympathomimetic amines. *Nature*, **214**, 597–598.

Lands, A. M., Luduena, F. P. & Buzzo, H. J. (1967b). Differentiation of receptors responsive to isoproterenol. *Life Sci.*, **8**, 2241–2249.

Lincoln, D. W. (1989). Luteinising hormone-releasing hormone. In *Endocrinology*, Vol.1, ed. DeGroot, L. J., 142–151. Philadelphia: W. B. Saunders.

Minneman, K. P., Hegstrand, L. R. & Molinoff, P. B. (1979). The pharmacological specificity of beta-1 and beta-2 adrenergic receptors in rat heart and lung *in vitro*. *Mol. Pharmac.*, **16**, 21–33.

Nials, A. T., Sumner, M. J. & Coleman, R. A. (1990). Quinprenaline, a long-acting β_2-adrenoceptor agonist *in vitro*—a comparison with salmeterol. *Br. J. Pharmac.*, Poster at December meeting.

Palmqvist, M., Bolder, B., Löwhager, O., Melander, B., Svedmyr, N. & Wahlander, L. (1989). Late asthmatic reaction prevented by inhaled albuterol and formoterol. *J. Allergy clin. Immunol.*, **83**, 244.

Robison, G. A., Butcher, R. W. & Sutherland, E. W. (1967). Adenyl cyclase as an adrenergic receptor. *Ann. N.Y. Acad. Sci.*, **139**, 703–723.

Scriabine, A., Moor, P. F., Iorio, L. C., Goldman, I. M., McShane, W. K. & Booher, K. D. (1968). Quinterenol, a new beta adrenergic stimulant. *J. Pharmac. exp. Ther.*, **162**, 60–69.

Sears, M. R., Taylor, D. R., Print, C. G., Lake, D. C., Qingqing, Li, Flannery, E. M., Yates, D. M., Lucas,

M. L. & Herbison, G. P. (1990). Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet*, **i**, 1391–1396.

Stephenson, R. P. (1956). A modification of receptor theory. *Br. J. Pharmac. Chemother.*, **11**, 379–393.

Strader, C. D., Sigal, I. S. & Dixon, R. A. F. (1989). Genetic approaches to the determination of structure-function relationships of G-protein-coupled receptors. *Trends Pharmac. Sci.*, December 1989 supplement. Sub-types of muscarinic receptors IV, pp 26–30.

Sutherland, E. W. & Rall, T. W. (1960). The relation of adenosine-3^1,5^1-phosphate and phosphorylase to the actions of catecholamines and other hormones. *Pharmac. Rev.*, **12**, 265–299.

Twentyman, O. P., Finnerty, J. P., Harris, A., Palmer, J. & Holgate, S. T. (1990). Protection against allergen-induced asthma by salmeterol. *Lancet*, **i**, 1338–1342.

Ullman, A. & Svedmyr, N. (1988). Salmeterol, a new long-acting inhaled β_2-adrenoceptor agonist: Comparison with salbutamol in adult asthmatic patients. *Thorax*, **43**, 647–678.

Vane, J. R. (1982). Adventures and excursions in bio-assay: the stepping stones to prostacyclin. Nobel lecture, 8 December 1982.

(Received 14 January 1991, accepted 18 January 1991)