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Molecular biology of receptors: implications for lung disease

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Receptors have a critical role in regulating lung function in health and disease.[1] Several such receptors have recently been sequenced, cloned, and genetically expressed. This opens up new vistas for investigating receptor regulation in pulmonary cells, for identifying receptor subtypes, and for understanding how disease may affect these regulatory processes. In the future it may also lead to new and more specific therapeutic approaches.

This article attempts to discuss some of the areas in which molecular approaches have been used to study receptor structure and regulation. At present, these novel techniques have hardly been applied to pulmonary tissues and lung disease, but it is clear that these are extraordinarily powerful tools that may now address previously unanswerable questions.

Receptor cloning

The genes for many neurotransmitter receptors have now been cloned. Several strategies have been used to isolate these DNA clones. Usually a DNA library prepared by partial digestion of genomic DNA from a tissue known to express the receptor is screened by using a labelled synthetic probe that is a part of the predicted DNA sequence of the receptor. Alternatively, a DNA probe is prepared with the aid of reverse transcriptase from messenger RNA (mRNA) extracted from a tissue expressing the receptor.

Once the receptor DNA or cDNA (that is, the complementary DNA produced from mRNA) is identified it can be inserted into a suitable vector (such as a plasmid) and then amplified (copied) in a suitable bacterial strain (usually a non-pathogenic strain of *Escherichia coli*), so that large numbers of exact copies may be made. This yields enough pure cDNA for the nucleotide sequence to be determined, leading to prediction of the amino acid sequence of the receptor.

Because there are structural similarities between receptors, such as G protein linked receptors (see below), a DNA probe from one receptor can be used to identify subtypes of the same receptor or even different receptors. For example, screening of a DNA library with a cross hybridising probe for a beta adrenoceptor led to the discovery of the serotonin (5-hydroxytryptamine IA) receptor.[2]

As many receptors are expressed only at very low levels in most tissues it may be difficult to obtain a sufficient quantity of the receptor for the initial sequencing necessary to prepare a probe. More recently, the polymerase chain reaction has been used to amplify the number of mRNA copies in a cell in order to make a series of probes that can then be used to screen a DNA library.[3]

The receptor DNA can also be inserted into an immortal cell line, resulting in its expression and thus providing a source of the receptor, so that the gene product can be characterised pharmacologically. The DNA may be microinjected directly into the large *Xenopus* (toad) oocyte, or transfected into a cultured mammalian cell that does not normally express the receptor by using a plasmid expression vector (ring of self replicating DNA into which the receptor DNA sequence of interest has been inserted), which after suitable manipulation inserts the DNA into the cultured cell genome. Cultured Chinese hamster ovary (CHO) and B-82 (a mouse L cell line) cells have been found to be very useful for studying neurotransmitter receptors because they lack these receptors but possess other elements such as G proteins and adenylyl cyclase, so that aspects of receptor coupling can be investigated. The cultured cells express the single receptor coded by the inserted DNA and this can be studied directly by radioligand binding and by measuring cellular responses, such as cyclic AMP concentrations or phosphoinositide hydrolysis. With the aid of a series of suitable agonists and antagonists the pure cloned receptor can then be characterised pharmacologically. Factors that influence the pure receptor can then be studied in detail without variations in metabolism and the interfering effects of related receptors.

Molecular structure of receptors

G PROTEIN LINKED RECEPTORS

Although neurotransmitter receptors vary widely in terms of specificity for agonists, molecular biological characterisation has recently revealed some common structural features. This is perhaps understandable because all these receptors interact with a guanine nucleotide regulatory protein (G protein). G proteins may stimulate (G_s) or inhibit (G_i) adenylyl cyclase and thus increase or decrease the intracellular concentration of cyclic AMP,[4] or they may stimulate a G protein that activates phospholipase C (G_p), resulting in phosphoinositide (PI) hydrolysis, which leads

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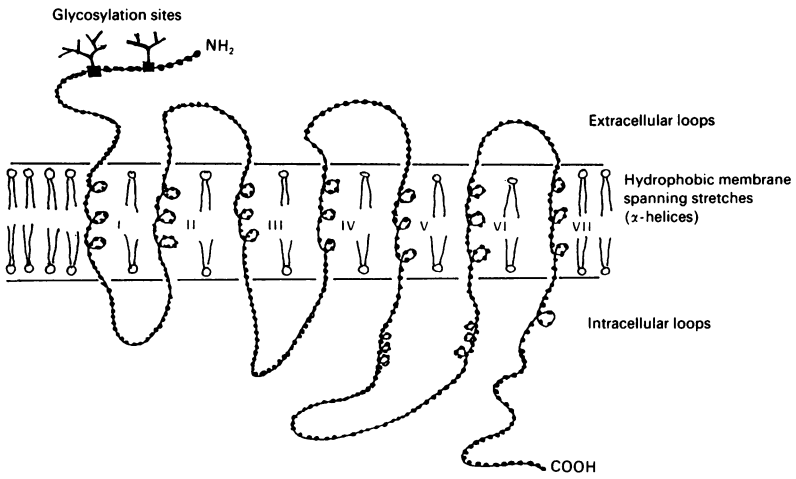

Figure 1 Molecular structure of G protein linked receptors. The amino acid sequence consists of seven transmembrane spanning helices (I–VII) with alternate extracellular and intracellular loops.

to the formation of inositol(1,4,5)trisphosphate, the intracellular messenger that releases calcium ions from internal stores.[5]

More than 20 G protein linked receptors have now been cloned and their sequences determined.[6 7] The first characterised receptor in this category was rhodopsin in light sensitive rods of the retina, which is linked to a unique G protein called transducin[8 9]; this has served as a useful model for other receptors in this group, which were cloned subsequently. Analysis of the amino acid sequence of rhodopsin has shown that seven hydrophobic (lipophilic) stretches of 20–25 amino acids are linked to hydrophilic regions of variable length. The most likely spatial arrangement of the receptor in the cell surface membrane is for the seven hydrophobic sections (each of which is in the form of an α helix) to span the cell membrane. The intervening hydrophilic sections are exposed alternately intracellularly and extracellularly with the amino (*N*) terminal end exposed to the outside and the carboxy (*C*) terminal end within the cytoplasm (fig 1). The extracellular regions of rhodopsin recognise the appropriate ligand (retinal) and the intracellular regions interact with the relevant G protein (transducin).

Several other G protein linked receptors have now been cloned and sequenced. These include $beta_1$,[10] $beta_2$,[11 12] $alpha_1$,[13] and $alpha_2$[14 15] adrenoceptors; muscarinic receptors[16–19]; and tachykinin receptors.[20 21] These receptors have the common feature of seven similar hydrophobic membrane spanning segments. There is also some sequence homology of the intracellular loops (which interact with various G proteins) but less similarity in the extracellular domain that recognises the different ligands. For example, there is a 50% homology between rat $beta_2$ adrenergic and muscarinic M_2 receptors.[22] There is also close homology between the same receptor in different species—for instance, there is 95% homology between rat and pig heart M_2 receptors.[22] These similarities support the view that the genes for G protein linked receptors form part of a superfamily that may have a common evolutionary origin.[7]

The receptors are generally 400–500 amino acids in length and the receptor DNA sequence consists of 2000–4000 nucleotide bases (2–4 kb). There is usually a striking lack of introns in the coding sequence of these receptors by comparison with genes for other proteins. The molecular weight of the cloned receptors predicted from the DNA sequence is 40 000–60 000 daltons (40–60 kDa), which is usually less than the molecular mass of the wild receptors when assessed by SDS polyacylamide gel electrophoresis. This discrepancy is due to glycosylation of the native receptor. For example, $beta_2$ receptors contain two sites for glycosylation on asparagine (Asn/N) residues near the amino terminus, and *N* glycosylation is estimated to account for 25–30% of the molecular mass of the native receptor. The functional significance of glycosylation is not yet clear[23]; it does not affect receptor affinity for ligand or coupling to G proteins but may be important for the trafficking of the receptor through the cell during down regulation.

STEROID RECEPTORS

Cortisteroids interact with intracellular steroid receptors rather than surface receptors. There is a family of steroid receptors that recognise different endogenous steroids, such as glucocorticosteroids, mineralocorticoids, androgens, and oestrogens. Indeed, these steroid receptors also appear to be governed by a gene superfamily that also covers thyroid hormone receptors.[24] Steroid receptors have now been cloned and their structures have been shown to differ.[25 26] There is, however, some homology between these receptors because they all interact with nuclear DNA, where they act as modulators of the transcription of a specific network of genes. For instance, corticosteroids switch on the transcription of the 37 kDa protein lipocortin, which may inhibit phospholipase A2,[27] though lipocortin is likely to be only one of several signal proteins induced by steroids.

The DNA binding domain of steroid receptors is rich in cysteine (Cys) residues. Formation of a complex with zinc folds the peptide chain into a finger shaped conformation, and the zinc is coordinated either by two Cys and two histidine or by four Cys residues. These "zinc fingers" appear to be essential for recognition of specific DNA sequences—the so called hormone response elements.[24] Understanding of the interactions between steroid receptors and DNA may enable synthetic agents to be developed that could mimic these responses. The steroid receptor-DNA interactions may be tissue specific and this opens up the possibility of developing more selective steroid like agents for therapeutic use, thereby reducing the potential hazards of systemic treatment.

Receptor subtypes

The existence of receptor subtypes is often first

indicated by differences in the potency of a series of agonists in different tissues. This could be due to differing proportions of co-existent receptor subtypes, or may indicate the existence of a novel receptor subtype. Molecular biology can resolve these uncertainties because molecular techniques clearly discriminate between different subtypes of receptor and show that they are encoded by different genes. Thus the human $beta_1$ receptor is clearly different from the $beta_2$ receptor in its amino acid sequence, with only a 54% homology,[28] and the neurokinin 1 (NK-1) receptor, which is selectively activated by substance P, has 48% homology with the NK-2 receptor, which is activated by the related mammalian tachykinin neurokinin A.[21]

Cross hybridisation in which a known receptor cDNA sequence is hybridised with a genomic library, has made it possible to detect previously unknown subtypes of a receptor. For example, an atypical beta receptor, which does not clearly fit into the $beta_1$ or $beta_2$ subtypes, has been suspected in adipose tissue and recently the "$beta_3$" receptor has been identified, cloned, sequenced and expressed.[29] The $beta_3$ receptor is clearly different from both $beta_1$ and $beta_2$ receptors (about 50% amino acid sequence homology). $Beta_3$ receptors appear to be important in regulation of metabolic rate and have not yet been detected in lung homogenates. Without the techniques of molecular biology this receptor would probably still remain undiscovered.

Molecular biology has been particularly useful in advancing our understanding of muscarinic receptors. It is now clear that there are at least three muscarinic receptor subtypes present in airways, which can be differentiated pharmacologically.[30-32] Five muscarinic receptors, however, have been cloned from rat and human tissues.[33] The m_1, m_2, and m_3 receptors correspond to the M_1, M_2, and M_3 receptors identified pharmacologically. The m_4 and m_5 receptors are previously unrecognised pharmacological subtypes that occur predominantly in the brain, for which no selective drugs have yet been developed. Related but as yet uncharacterised genes could represent additional subtypes and up to nine subtypes have been predicted in the rat.[34] The reason for so many different subtypes of a receptor that recognises a single agonist is still not certain, but they seem likely to be linked to different intracellular pathways and the regulation of the intracellular portion of the amino acid sequence may be unique to each subtype. The m_1, m_3, and m_5 receptors stimulate phosphoinositide hydrolysis through a pertussis insensitive G protein, whereas m_2 and m_4 receptors inhibit adenylyl cyclase via G_1.[28] The picture is complicated further by the fact that, at higher agonist concentrations, m_2 and m_4 receptors can also stimulate phosphoinositide hydrolysis.[35] Possibly, the difference in protein structure reflects regulation at a transcriptional level from DNA through different promoters, leading to variations in tissue or developmental expression, or to differences at a post-translational level, allowing regulation by intracellular mechanisms such as phosphorylation at critical sites on intracellular loops.

Several other subtypes of known receptors are likely to be identified in the future by the technique of cross hybridisation. This should have important implications for the development of more selective receptor agonists. The potential clinical importance of muscarinic receptor subtypes in airways is already apparent,[30] and the recognition of different receptor subtypes has prompted the search for M_3 selective antagonists to treat airway obstruction. Similarly, for alpha receptors additional subtypes have been identified by molecular cloning techniques. There appear to be at least two $alpha_2$ receptors—the $alpha_2$ receptors or platelets being different from those found in the kidney.[36]

Molecular approaches may be particularly valuable in the study of peptide receptors, where selective agonists and antagonists have been difficult to develop. If distinct receptors are discovered (which has not been possible by conventional pharmacological techniques), this may lead to the development of selective agonists and antagonists. This has been the problem with bradykinin receptors in airways which do not clearly fit into the expected bradykinin B_2 receptor class (because B_2 receptor antagonists are not very effective), and it has been suggested that B_3 receptors may exist.[37] Similarly, for platelet activating factor there is evidence for more than one subtype of receptor.[38,39] The potential diversity of receptors is obviously an important consideration in the design of agonists and antagonists for the treatment of lung diseases, but the coexistence of receptor subtypes raises the possibility that more selective drugs may be developed in the future.

Site directed mutagenesis

Once the DNA sequence of receptor genes is known single nucleotide bases can be substituted at different positions in the sequence, thus producing mutant receptor with predetermined alterations in its of amino acid sequence. This is carried out by substituting the codon for one amino acid with that for another in a synthetic oligonucleotide, and creating a mutant gene which, when expressed, produces a receptor with minor specific changes. This technique of site directed mutagenesis has proved to be invaluable in elucidating receptor structure and function. Replacement of the Cys residues at positions 106 and 184 in the second and third extracellular domain in the hamster lung $beta_2$ receptor has shown that they are important in maintaining the correct tertiary structure (three dimensional shape) of the receptor in the cell membrane by the formation of a disulphide link between the two loops. Substitution of either of these Cys residues with isoleucine (Ile) impairs beta receptor ligand binding affinity.[40] Similarly, substitution of either of the adjacent Cys residues at positions 190 and 191 on the third cytoplasmic loop of the human $beta_2$ receptor substantially reduces binding affinity and coupling to adenylyl cyclase.[41] Interestingly, the Cys

residues on the second and third extracellular loops are highly conserved among receptors, which suggests that they may be important in stabilising the three dimensional structure.

One of the most intriguing questions is the location of the ligand binding site and the means by which an agonist changes the receptor so that it can interact with the appropriate G protein. The ligand binding domain of the beta receptor has been carefully characterised by selective deletion of sections of the gene encoding hydrophobic and hydrophilic domains of the receptor protein, expressing the mutant receptor genes in mammalian cell lines, and determing the binding and functional characteristics of the mutant receptor. These elegant studies have shown that regions that form junctions between the transmembrane hydrophobic segments and the extramembranous loops result in altered protein folding. Curiously, most of the extracellular portions of the receptor can be deleted without affecting binding, which implicates regions within the hydrophobic membrane portion as being critical for binding.[42] Site directed mutagenesis of single amino acids of the $beta_2$ receptor has indicated that substitution of neutral amino acids for aspartate (Asp) residues in the second and third transmembrane segments affect agonist binding affinity, but do not affect Gs activation. The importance of Asp residues may relate to the fact that they are believed to bind to the protonated group of catecholamines.[43–45] Interestingly, equivalent Asp residues are conserved in all other adrenergic receptors, which all bind ligands with a charged amine group.

To explain how adrenergic receptors are selective for catecholamines, however, differences between G protein linked receptors that recognise different agonists must be examined. Site directed mutagenesis of serine (Ser) residues, which may form a hydrogen bond with the catechol ring, suggests that Ser residues in the fifth transmembrane helix are important. These studies indicate that a ligand binding pocket may fit between the third to sixth transmembrane helix.[40]

The reason why $beta_1$ receptors preferentially bind noradrenaline, and $beta_2$ receptors prefer adrenaline (which is more bulky), is not completely understood. Recent studies with chimeric receptors, which are a combination of the sequences of $beta_1$ and $beta_2$ receptors, indicate that the sequences of amino acids in transmembrane helices 4 and 5 are critical and probably determine the shape of the binding pocket.[46]

The intracellular loops are located on the inner surface of the plasma membrane that interacts with G proteins. Deletion mutagenesis studies, in which stretches of the amino acid sequence are removed, indicate that the third intracellular loop is critical for interaction between $beta_2$ receptors and Gs.[47] This loop contains a sequence of amino acids that include charged amino acids in the form of a helix. A similar sequence is found in all G protein linked receptors and the third cytoplasmic loop is also present in muscarinic receptors.[34] The specificity for the particular G protein (G_s, G_i, or G_p) is probably determined by the remaining intracellular loops.

Quantification and localisation of receptor transcription

NORTHERN BLOTS

Transcription of the genes coding for receptors leads to the formation of specific mRNA, which leaves the nucleus and becomes located on ribosomes, where amino acids are assembled in sequence to form the receptor protein. Total RNA, which includes mRNAs, can be extracted from tissues and specific receptor mRNA can then be identified by hybridisation with a radiolabelled cDNA probe that contains the unique receptor coding sequence. This probe may be the full receptor sequence or may be relatively short (oligoprobe) and is labelled by incorporating either [^{35}S]uracil or [^{32}P]cytosine bases into the sequence.

In Northern blot analysis the RNA is run on a size separation gel, and then hybridised with the labelled cDNA (or complementary RNA) probe to show whether there is a labelled band that corresponds to the receptor mRNA. Specific mRNA represents only a small percentage of total RNA and may be difficult to detect, especially when the amounts are small (which is usually the case for receptor mRNA). The mRNA that is transcribed, however, is characterised by a long polyadenine (poly(A)) tail and it is therefore possible to extract such mRNA selectively with column chromatography by using the complementary polydeoxythymidine (poly(dT)). The poly(A) tail of mRNA hybridises with the complementary poly(dT)) on the column and is thus retained while the other RNA molecules pass through. The mRNA can subsequently be harvested for study.

With this approach the distribution of muscarinic receptor subtype mRNAs has been determined in different tissues. This indicates that both m_2 and m_3 receptors are found in porcine and rat tracheal smooth muscle.[48] $Beta_2$ receptor mRNA has also been detected in human and rat lung[49] and in guinea pig lung mast cells.[50] The amount of radioactivity in the labelled band can be quantified by laser densitometry to give a semiquantitative estimate of the amount of mRNA present. This makes it possible to assess changes in gene transcription of a particular receptor. Results are usually expressed in comparison with those of a gene that shows consistent expression (for example, β actin), to overcome the variability in RNA purification.

As many receptors are present in relatively small numbers (by comparison with other proteins) there may be few mRNA copies present in a particular cell. By using the polymerase chain reaction it may be possible to amplify the number of copies in order the detect the mRNA for a particular receptor subtype.[3] This technique is potentially relevant to investigating the role of receptors in lung disease because it may be possible to detect and study receptor mRNA when only a small

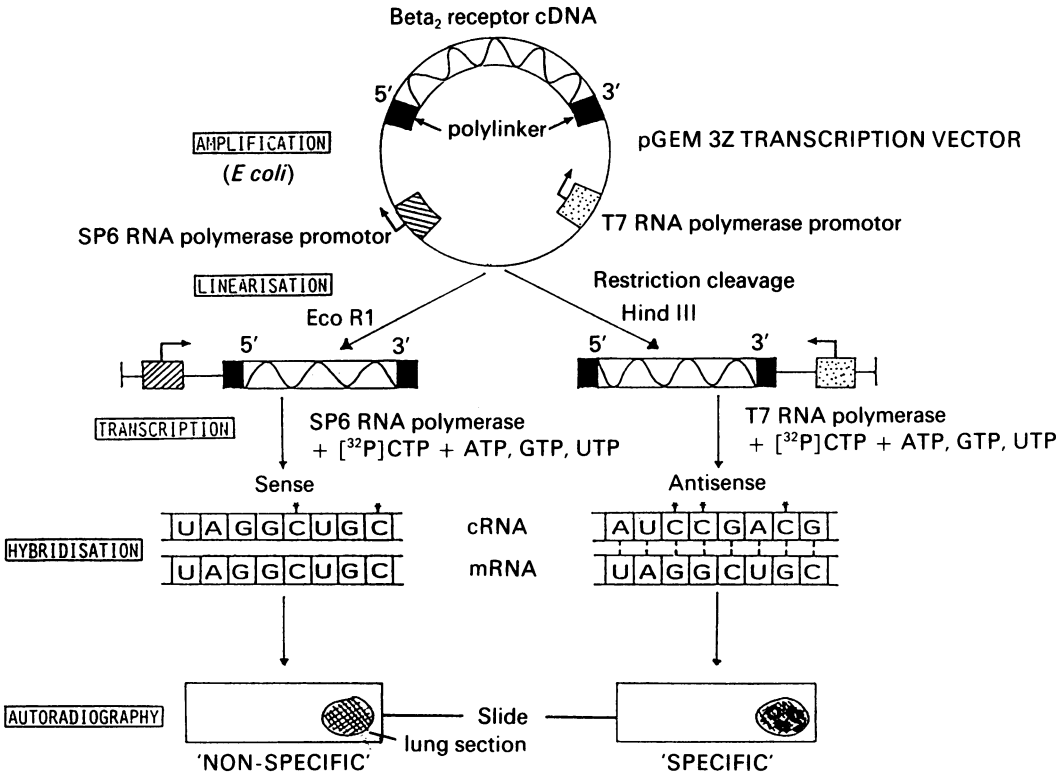

Figure 2 In situ hybridisation to localise mRNA for beta$_2$ receptors. The complementary DNA (cDNA) sequence encoding the beta$_2$ adrenergic receptor is inserted into a plasmid vector and amplified. The sequence is then cut by specific restriction enzymes and the linearised cDNA used to generate both a sense and an antisense labelled cRNA with the aid of the appropriate RNA polymerase with nucleotides, which include [^{32}P]labelled CTP. The sense or antisense probe generated by each RNA polymerase depends on the original orientation of the cDNA in the plasmid vector. The labelled probe (riboprobe) is then hybridised with beta receptor mRNA in lung tissue sections, and autoradiography is used to locate the sites of mRNA.

amount of tissue is available (for example, from a bronchial or lung biopsy specimen, from lavaged cells, or from bronchial brushings).

IN SITU HYBRIDISATION

In situ hybridisation is a particularly useful application of molecular biology that is capable of determining the distribution of mRNA in tissue sections. As the amount of mRNA for receptors is present in only low concentrations, a special, highly sensitive technique must be used. This depends on the construction of labelled RNA probes (riboprobes) that are complementary to mRNA and therefore have greater affinity for hybridising with the specific mRNA in the cell than the relevant DNA (fig 2). Autoradiography shows the tissue distribution of receptor mRNA, and therefore the sites of gene transcription of the receptor. With a full length or long DNA probe and thus cRNA sequence the probe can be labelled at many sites and thus to the radioactivity of the probe increased, so that there is a stronger signal. There may, however, be false positive results and suitable controls have to be included. This requires the construction and use of a labelled *sense* probe (which has a sequence identical to that of mRNA), whereas the riboprobe is an *antisense* probe, complementary to the mRNA with which it hybridises (fig 2).

With this approach the distribution of beta$_2$ receptor mRNA has been seen in rat and human lung and compared with the distribution of beta$_2$ receptor binding sites.[51] The localisation of receptor mRNA corresponds to the distribution of beta$_2$ receptors as expected[52] but there are discrepancies between the relative density of mRNA and of receptors in certain cells. For example, in airway smooth muscle there is a very high density of beta receptor mRNA, whereas the density of the beta receptors is relatively low; this suggests either that the rate of receptor synthesis is high, and that there is a rapid turnover of receptors, or that the stability of mRNA is low. This may explain why it is difficult to down regulate beta receptors in airway smooth muscle, and therefore to detect tachyphylaxis to the bronchodilator action of beta agonists. By contrast, in the alveolar walls there is a low level of mRNA but a very high receptor density, which may indicate a low receptor turnover, and this would be consistent with the fact that down regulation is readily produced in lung parenchyma. Preliminary studies have also shown that muscarinic M_3 receptor mRNA is localised to the smooth muscle and submucosal glands of human and guinea pig airway smooth muscle (J C Mac, J Baraniuk, P J Barnes, unpublished observations), which is the site of M_3 receptors determined by receptor autoradiographic mapping.[53]

In situ hybridisation is now possible for all receptors that have been cloned. These receptors include corticosteroid receptors (glucocorticoid receptor mRNA has been localised in brain tissue,[54] so that localisation in lung cells should also be possible. In situ hybridisation is a very powerful took, which should provide important insights into the genetic transcription of receptors in different cells of the lung, including inflammatory cells isolated by bronchoalveolar lavage. It may also be applied to bronchial and lung biopsy tissue, providing information about receptor regulation in disease.

Regulation of receptor transcription

Perhaps one of the most useful applications of molecular biology to receptor pharmacology will be in understanding further how receptors

may be regulated in health and disease, though few such studies have so far been reported.

DESENSITISATION

Tachyphylaxis or densensitisation occurs with several receptors when they are exposed to an agonist. This phenomenon has been studied in some detail with beta$_2$ receptors and comprises several processes,[55] which include down regulation, in which receptors disappear from the cell surface, and phosphorylation, which uncouples the receptor from Gs via the action of an enzyme called beta adrenergic receptor specific kinase.[56] The site of this phosphorylation appears to be on the Ser/Thr rich region of the third intracellular loop and the carboxy-terminal tail, because their replacement reduces the rate of desensitisation.[57] In a cultured hamster cell line down regulation of beta$_2$ receptors results in a rapid decline in the steady state level of beta$_2$ receptor mRNA.[58] This suggests that down regulation is achieved in part either by inhibiting the gene transcription of receptors or by increased post-transcriptional catabolism of the mRNA in the cell. With the use of actinomycin D to inhibit transcription it has been found that beta$_2$ receptor mRNA stability is substantially reduced in these cells after exposure to beta agonists. Furthermore, by isolating nuclei and performing a nuclear run on transcription assay beta agonist exposure is seen not to alter receptor gene transcription.[59] Whether this also applies to beta$_2$ receptors in various lung cells has not been reported.

STEROID MODULATION

Certain G protein linked receptors are also influenced by corticosteroids. Thus rat pulmonary beta receptors are increased in density by pretreatment with corticosteroids,[60] and corticosteroids increase the expression of beta receptors in rabbit fetal lung.[61] Steroids also prevent the desensitisation and down regulation of beta receptors on human leuckocytes.[62] Corticosteroids increase the steady state level of beta$_2$ receptor mRNA in cultured hamster smooth muscle cells, thus indicating that steroids may increase beta receptor density by increasing the rate of gene transcription.[59 63] The increase in mRNA occurs rapidly (within one hour), preceding the increase in beta receptors, and then declines to a steady state level about twice normal. The mechanism by which steroids interact with the beta receptor gene is not yet understood but the cloned beta$_2$ receptor gene contains three potential glucocorticoid responsive elements, which have the sequence (T/A)GT(T/CCT).[11 12]

ONTOGENY

Another area in which the molecular biology of receptors may be relevant is in the studying of the development of receptors in the lung. Pulmonary beta receptors increase in density during fetal development and during the postnatal period.[64] With the use of molecular probes it should now be possible to study the development of gene transcription and of post-transcriptional processing of these receptors. This may give insights into how expression of certain receptors may be altered during development.

LUNG DISEASE

There has been considerable interest in whether pulmonary adrenoceptors may be abnormal in disease.[65] There is some evidence that beta receptors may be dysfunctional in asthmatic airways,[66 67] which may be due to uncoupling of receptors owing to phosphorylation by activation of protein kinase C, which could result from phosphoinositide hydrolysis stimulated through inflammatory mediator receptors.[68] Whether inflammation itself may affect beta receptor transcription may now be determined with the help of suitable probes. A defect in M_2 receptors on cholinergic nerves in asthmatic airways has also been proposed,[68] and whether there is any defect in M_2 receptor synthesis could be determined by using the relevant probe.

Conclusions

The potential impact of molecular biology on the understanding of the structure and function of pulmonary receptors is vast. Use of these novel techniques should provide insight into the structure of the neurotransmitter, inflammatory mediator, and steroid receptors relevant to lung disease, and will give further understanding of abnormalities that may occur in disease. In the future this new knowledge should lead to advances in treatment, because understanding receptor structure and function will aid the development of new agonists or antagonists (which may be particularly valuable in the case of peptide receptors) and may also lead to novel drugs that interact with the transcription or post-translational processing of receptors.

1 Barnes PJ. Airway receptors. *Postgrad Med J* 1989;65: 532–42.
2 Fargin A, Raymond JR, Lohse MJ, *et al.* The genomic clone G-21 which resembles a beta-adrenergic receptor sequence encodes the 5-HT1A receptor. *Nature* 1988;335: 358–60.
3 Watson SP, James W. PCR and the cloning of receptor subtype genes. *Trends Pharmacol Sci* 1989;10:346–8.
4 Gilman AG. G-proteins: transducers of receptor-generated signals. *Annu Rev Biochem* 1987;56:615–49.
5 Berridge MJ. Inositol trisphosphate and diacylglycerol: two interacting second messengers. *Annu Rev Biochem* 1987; 56:159–93.
6 Dohlman HG, Caron MG, Lefkowitz RJ. A family of receptors coupled to guanine nucleotide regulatory proteins. *Biochemistry* 1987;26:2656–64.
7 Venter JC, Fraser CM, Kerlavage AR, Buck MA. Molecular biology of adrenergic and muscarinic cholinergic receptors: a perspective. *Biochem Pharmacol* 1989;38:1197–208.
8 Nathans J, Hogness DS. Isolation and nucleotide sequence of the gene encoding human rhodopsin. *Proc Natl Acad Sci USA* 1984;81:4851–5.
9 Martin RL, Wood C, Baehr W, Applebury ML. Visual pigment homologies revealed by DNA hybridization. *Science* 1986;232:1266–9.
10 Frielle T, Collins S, Daniel KW, Caron MG, Lefkowitz RJ, Kobilka BK. Cloning of the cDNA for the B1-adrenergic receptor. *Proc Natl Acad Sci USA* 1987;84:7920–4.
11 Dixon RAF, Kobilka BK, Strader D, *et al.* Cloning of the gene and cDNA for mammalian β-adrenergic receptor and homology with rhodopsin. *Nature* 1986;321:75–9.
12 Chung F-Z, Lentes K-U, Gocayne J, *et al.* Cloning and sequence analysis of the human brain B-adrenergic receptor. *FEBS Lett* 1987;211:200–6.
13 Cottecchia S, Schwinn DA, Randall RR, Lefkowitz RJ, Caron MG, Kobilka BK. Molecular cloning and expression of the cDNA for the hamster α_1-adrenergic receptor. *Proc Natl Acad Sci USA* 1988;85:7159–63.
14 Kobilka BK, Matsui H, Kobilka TS, *et al.* Cloning, sequencing and expression of the gene coding for the human

platelet α_2-adrenergic receptor. *Science* 1987;**238**:650–6.
15 Fraser CM, Arakawa S, McCrombie WR, Venter JC. Cloning, sequence analysis, and permanent expression of human α_2-adrenergic receptor in Chinese hamster ovary cells. *J Biol Chem* 1989;**264**:11754–61.
16 Kubo T, Fukuda K, Mikami A, *et al.* Cloning, sequencing and expression of complimentary DNA encoding the muscarinic acetylcholine receptor. *Nature* 1986;**323**: 411–6.
17 Peralta EG, Winslow JW, Peterson GL, *et al.* Primary structure and biochemical properties of an M_2 muscarinic receptor. *Science* 1987;**236**:600–5.
18 Bonner TI, Buckley NJ, Young AC, Brann MR. Identification of a family of muscarinic acetylcholine receptor genes. *Science* 1987;**237**:527–32.
19 Bonner TI, Young AC, Brann MR, Buckley NJ. Cloning and expression of the human and rat m_5 muscarinic acetylcholine receptor genes. *Neuron* 1988;**1**:403–10.
20 Matsu Y, Nakayama K, Tamaki H, Harada Y, Kuno M, Nakanishi S. cDNA cloning of bovine substance-K receptor through oocyte expression system. *Nature* 1987;**329**: 836–8.
21 Yokota Y, Sasai Y, Tanaka K, *et al.* Molecular characterization of a functional cDNA probe for rat substance P receptor. *J Biol Chem* 1989;**264**:17649–52.
22 Gokayne J, Robinson DA, Fitzgerald MG, *et al.* Primary structure of rat cardio B-adrenergic and muscarinic receptors obtained by automated DNA sequence analysis: further evidence for a multigene family. *Proc Natl Acad Sci USA* 1987;**84**:8296–300.
23 George ST, Ruolto AE, Malbon CC. *N*-glycosylation in expression and function of β_2-adrenergic receptors. *J Biochem* 1986;**261**:16559–664.
24 Evans RM. The steroid and thyroid hormone receptor superfamily. *Science* 1988;**247**:889–95.
25 Miesfeld R, Okret S, Wikstrom A-C, Wrange O, Gustafson J-A, Yamamoto KR. Characterization of a steroid hormone receptor gene and mRNA in wild-type and mutant cells. *Nature* 1984;**312**:779–82.
26 Thompson BE. The structure of the human glucocorticoid receptor and its gene. *J Steroid Biochem* 1987;**27**:105–8.
27 Flower RJ. Lipocortin and the mechanism of action of the glucocorticosteroids. *Br J Pharmacol* 1988;**94**:987–1015.
28 Lefkowitz RJ, Caron MG. Adrenergic receptors. Models for the study of receptors coupled to guanine nucleotide regulatory proteins. *J Biol Chem* 1988;**263**:4993–6.
29 Emorine LJ, Marullo S, Briend-Sutren M-M, *et al.* Molecular characterization of the human B3-adrenergic receptor. *Science* 1989;**245**:1118–21.
30 Barnes PJ. Muscarinic receptor subtypes: implications for lung disease. *Thorax* 1989;**44**:161–7.
31 Barnes PJ. Muscarinic receptor in airways: recent developments. *J Appl Physiol* (in press).
32 Maclagan J, Barnes PJ. Muscarinic pharmacology of the airways. *Trends Pharmacol Sci* 1989;**10**(suppl):88–9.
33 Buckley NJ, Bonner TI, Buckley CM, Brann MR. Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-KI cells. *Mol Pharmacol* 1989;**35**: 469–76.
34 Bonner TI. New subtypes of muscarinic acetylcholine receptors. *Trends Pharmacol Sci* 1989;**10**(suppl):11–5.
35 Ashkenazi A, Winslow JW, Peralta EG, *et al.* A M_2-muscarinic receptor coupled to both adenylyl cyclase and phosphoinositide turnover. *Science* 1987;**238**:672–5.
36 Regan JW, Kobilka TS, Yang-Feng TL, Caron MG, Lefkowitz RJ, Kobilka BK. Cloning and expression of a human kidney cDNA for an α-adrenergic receptor subtype. *Proc Natl Acad Sci USA* 1988;**85**:6301–5.
37 Farmer SG, Burch RM, Meeker SA, Wilkins DE. Evidence for a pulmonary B_3-bradykinin receptor. *Mol Pharmacol* 1989;**36**:1–8.
38 Kroegel C, Yukawa T, Westwick J, Barnes PJ. Evidence for two platelet activating factor receptors on eosinophils: dissociation between PAF induced intracellular calcium mobilization, degranulation and superoxide anion generation. *Biochem Biophys Res Commun* 1989;**162**:511–21.
39 Dent G, Ukena D, Barnes PJ. PAF receptors. In: Barnes PJ, Page CP, Henson PM, eds. *PAF and human disease.* Oxford: Blackwell (in press).
40 Strader CD, Sigal IS, Dixon RAF. Structural basis of β-adrenergic receptor function. *Faseb J* 1989;**3**:1825–32.
41 Fraser CM. Site-directed mutagenesis of β-adrenergic receptors. *J Biol Chem* 1989;**264**:9266–70.
42 Dixon RAF, Sigal IS, Rands E, Register RB, Candelore MR, Blake AD, Strader CD. Ligand binding to the β-adrenergic receptor involves its rhodopsin-like core. *Nature* 1987;**326**:73–7.
43 Strader CD, Sigal IS, Candelore MR, Rands E, Hill WS, Dixon RAF. Conserved aspartin acid residues 79 and 113 of the β-adrenergic receptor have different roles in receptor function. *J Biol Chem* 1988;**263**:10267–71.
44 Fraser CM, Chung F-Z, Wang C-D, Venter JC. Site-directed mutagenesis of human β-adrenergic receptors: substitution of aspartic acid-130 by asparagine produces a receptor with high-affinity agonist binding that is uncoupled for adenylylate cyclase. *Proc Natl Acad Sci USA* 1988;**85**:5478–82.
45 Chung F-Z, Wang C-D, Potter PC, Venter JC, Fraser CM. Site-directed mutagenesis and continuous expression of human β-adrenergic receptors. *J Biol Chem* 1988;**263**: 4052–5.
46 Kobilka BK, Kobilka TS, Daniel K, Regan JW, Caron MG, Lefkowitz RJ. Chineric α_2- and β_2-adrenergic receptors: delineation of domains involved in effector coupling and ligand specificity. *Science* 1988;**240**:1310–6.
47 O'Dowd BF, Hnatowich M, Regan JW, Leader WM, Caron MG, Lefkowitz RJ. Site-directed mutagenesis of the cytoplasmic domains of the human β_2-adrenergic receptor. *J Biol Chem* 1988;**263**:15985–92.
48 Maeda A, Kubo T, Mishina M, Numa S. Tissue distribution of mRNAs encoding muscarinic acetylcholine receptor subtypes. *FEBS Lett* 1988;**239**:339–42.
49 Hamid Q, Mak JCW, Sheppard MN, Corrin B, Barnes PJ. Expression of β_2-adrenergic receptor gene in rat and human lung. *Thorax* 1989;**44**:835–6.
50 Arbabian M, Graziano FM, Jicinsky J, Hadcock J, Malbon C, Rucho A. Photoaffinity labeling of the guinea pig pulmonary mast cell beta receptor. *Am J Respir Cell Mol Biol* 1989;**1**:351–9.
51 Mak JCW, Hamid Q, Sheppard MN, Corrin B, Venter JC, Barnes PJ. Localisation of beta-2 adrenoceptor messenger RNA in human and rat lung using in situ hybridization: correlation with receptor autoradiography [abstract]. *Am Rev Respir Dis* (in press).
52 Carstairs JR, Nimmo AJ, Barnes PJ. Autoradiographic visualization of beta-adrenoceptor subtypes in human lung. *Am Rev Respir Dis* 1985;**132**:541–7.
53 Mak JCW, Barnes PJ. Autoradiographic visualization of muscarinic receptor subtypes in human and guinea pig lung. *Am Rev Respir Dis* (in press).
54 Aronson M, Fuxe K, Dong Y, Agnati LF, Okret S, Gustafsson J-A. Localization of glucocorticoid receptor mRNA in the male rat brain *in situ* hybridization. *Proc Natl Acad Sci USA* 1988;**85**:9331–5.
55 Stiles GL, Caron M, Lefkowitz RJ. β-Adrenergic receptors. *Pharmacol Rev* 1984;**64**:661–743.
56 Benovic JL, Strasser RH, Benovic JL, Daniel K, Lefkowitz RJ. Beta-adrenergic receptor kinase: identification of a novel protein kinase that phosphorylates the agonist-occupied form of the receptor. *Proc Natl Acad Sci USA* 1986;**83**:2797–801.
57 Bouvier MW, Hausdorff A, De Blasi A, *et al.* Removal of phosphorylation sites from the β-adrenergic receptor delays the onset of agonist-promoted desensitization. *Nature* 1988;**333**:370–3.
58 Bouvier M, Collins S, O'Dowd BF, *et al.* Two distinct pathways for cAMP-mediated down-regulation of the β_2-adrenergic receptor. *J Biol Chem* 1989;**264**:16786–792.
59 Hadcock JR, Wang HY, Malbon CC. Agonist-induced destabilization of β-adrenergic receptor mRNA: attenuation of glucocorticoid-induced up-regulation of β-adrenergic receptors. *J Biol Chem* 1989;**264**:19928–33.
60 Mano K, Akbarzadeh A, Townley RG. Effect of hydrocortisone on beta-adrenergic receptors in lung membranes. *Life Sci* 1979;**25**:1925–30.
61 Barnes PJ, Jacobs MM, Roberts JM. Glucocorticoids preferentially increase fetal alveolar beta-receptors: autoradiographic evidence. *Paediatr Res* 1984;**18**: 1191–4.
62 Davis AO, Lefkowitz RJ. *In vitro* desensitization of beta-adrenergic receptors in human neutrophils. Attenuation by corticosteroids. *J Clin Invest* 1983;**71**:565–71.
63 Collins S, Caron MG, Lefkowitz RJ. β_2-adrenergic receptors in hamster smooth muscle cells are transcriptionally regulated by glucocorticosteroids. *J Biol Chem* 1988; **263**:9067–70.
64 Giannopoulos G. Identification and ontogeny of β-adrenergic receptors in fetal rabbit lung. *Biochem Biophys Res Commun* 1980;**95**:388–96.
65 Barnes PJ. State of art. Neural control of human airways in health and disease. *Am Rev Respir Dis* 1986;**134**:1289–314.
66 Goldie RG, Spina D, Henry PJ, Lulich KM, Paterson JW. *In vitro* responsiveness of human asthmatic blockers to carbachol, histamine, β-receptor agonists and theophylline. *Br J Clin Pharmacol* 1986;**22**:669–76.
67 Cerrina J, Ladurie ML, Labat C, Raffestin B, Bayol A, Brink C. Comparison of human bronchial muscle responses to histamine in vivo with histamine and isoprotenerol agonists in vitro. *Am Rev Respir Dis* 1986;**134**:57–61.
68 Grandordy B, Rhoden K, Barnes PJ. Effects of protein kinase C activation on adrenoceptors in airway smooth muscle. *Biochem Biophys Res Commun* (in press).
69 Minette PAH, Lammers J, Dixon CMS, McCusker MT, Barnes PJ. A muscarinic agonist inhibits reflex bronchoconstriction in normal but not in asthmatic subjects. *J Appl Physiol* 1989;**67**:2461–5.