β_2 adrenoceptor polymorphisms: are they clinically important?

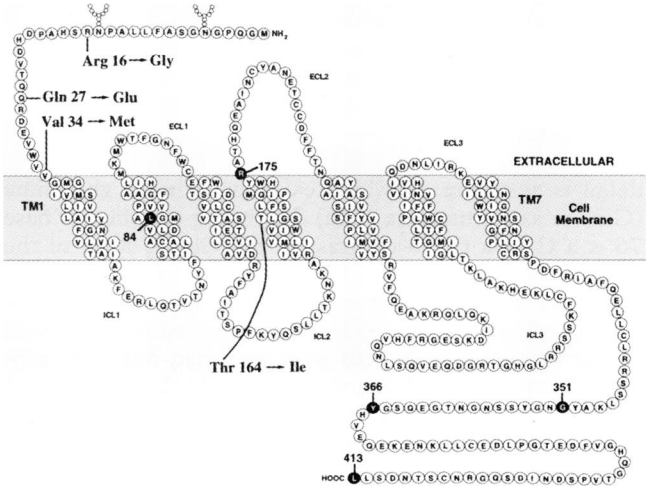
β_2 adrenoceptor agonists such as salbutamol, terbutaline, and salmeterol remain the most important bronchodilator agents used in the treatment of asthma and chronic airways obstruction. Following suggestions that overuse of β_2 agonists may worsen asthma control, an extensive research effort has investigated the possibility that long term exposure to β_2 agonists may result in tachyphylaxis to either the bronchodilator effects of these drugs or to the protective effect of β_2 agonists upon airway reactivity. An implicit assumption in this work has been that the mechanisms underlying the response of individuals to these drugs are common to all people. The effects of β_2 agonists in the airways are mediated by binding of the drug to the β_2 adrenoceptor. A number of common variants (polymorphisms) of the β_2 adrenoceptor have recently been described which alter the behaviour of the receptor following agonist exposure.[1] Hence, individual variability to the effects of β_2 agonists in the airways may exist and, indeed, it has been suggested that these polymorphisms may also be associated with asthma severity. The purpose of this editorial is to review the evidence that β_2 adrenoceptor polymorphisms are relevant to airway disease.

β_2 agonists have two main effects on the airways. Firstly, they rapidly reverse bronchoconstriction by relaxing airway smooth muscle.[2] The mechanisms underlying this response are probably related to a number of intracellular events, most of which are mediated by an increase in cyclic AMP levels within airway smooth muscle cells. The β_2 adrenoceptor is coupled via a G protein (Gs) to adenylyl cyclase, the enzyme responsible for the production of cyclic AMP, and hence stimulation of the β_2 adrenoceptor leads to an increase in intracellular cyclic AMP levels. Cyclic AMP activates protein kinase A, which in turn phosphorylates key regulatory proteins involved in the control of muscle tone. It has also been suggested recently that some of the relaxant response to β_2 agonists may be mediated through cyclic AMP independent mechanisms involving direct interaction of Gsα (the α subunit of the G protein Gs) with potassium channels which are present in the airway smooth muscle cell membrane.[3] The second important effect of β_2 agonists on the airways is that they protect against bronchoconstrictor challenge.[2] The exact mechanism underlying this response remains uncertain but may involve actions on a range of different cell types in the airways.

Following prolonged stimulation, β_2 receptors become uncoupled from their intracellular signalling pathways and are eventually lost from the cell surface, processes termed desensitisation and downregulation, respectively.[4] If these processes occur in the airways of an asthmatic patient a reduction in the effectiveness of β_2 agonists would be expected. Tachyphylaxis to the bronchodilator effects of β_2 agonists has been difficult to demonstrate in mild asthmatics,[5] although β_2 adrenoceptor dysfunction has been seen in in vitro studies of tissue or cells obtained from patients with severe asthma.[6–8] Tachyphylaxis to the protective effect of β_2 agonists on bronchial reactivity when measured by non-specific challenge has been seen in a number of studies.[9] In addition, rebound increases in bronchial reactivity upon cessation of regular β_2 agonist treatment have also been found,[10] although this has not been seen in all studies. However, taken together, there is a reasonable amount of evidence to suggest that downregulation and/or uncoupling of β_2 adrenoceptors occurs in the airways following prolonged exposure to β_2 agonists. The main clinical interest in β_2 receptor polymorphisms lies in the possibility that these polymorphisms may alter the way in which the receptor downregulates in the airways. Thus, β_2 adrenoceptor polymorphisms could potentially affect airway responses by altering the expression and coupling of β_2 receptors in airway cells.

The human β_2 adrenoceptor gene is situated on the long arm of chromosome 5 and codes for an intronless gene product of just over 1200 base pairs.[11] The initial studies on the β_2 adrenoceptor performed by Liggett's group in Cincinnati[1] identified a total of nine different polymorphisms (figure). All of these differed from the accepted wild type sequence by a single base change at different positions in the coding sequence of the gene. Because of redundancy in the amino acid code, a number of these polymorphisms are clinically silent – that is, although there was a base change in the coding sequence for the β_2 adrenoceptor gene the resultant receptor had the same amino acid squence as the wild type form. However, four polymorphisms resulting from single base changes were identified which altered the amino acid sequence of the receptor protein, all resulting in single amino acid substitutions. The obvious question that needs to be addressed is therefore: are these merely polymorphisms which are functionally irrelevant (such as can be found in many other genes) or do these amino acid substitutions confer functionally important changes on the receptor? Three of these polymorphisms have now been studied in some detail, and all three appear to alter the functional properties of the receptor such that the airways of individuals with these different forms of the receptor might be expected to behave differently when exposed to circulating catecholamines or exogenously applied β_2 agonists.

In order to study the effects of these polymorphisms on receptor coupling, a number of different approaches have



Predicted conformation of the human β_2 adrenoceptor showing the position of the known polymorphisms within the receptor. Clinically silent polymorphisms (i.e. those that do not alter the amino acid sequence) are shown in black, whereas polymorphisms which alter the amino acid sequence are labelled. Reproduced from Reishaus et al[1] with permission.

been necessary. Initial work made use of transfected cell systems, where the different forms of the receptor were generated by site directed mutagenesis and subcloned into a suitable expression vector. Stably transfected cell lines were then generated, expressing the different forms of the receptor. The potential problem with this type of experimental approach is that the levels of expression of the receptor protein may be different from cells which naturally express the receptor and, in addition, expression of the receptor gene will inevitably be regulated in a different manner because the gene elements controlling transcription are very different from those present upstream of the β_2 adrenoceptor gene on chromosome 5. The next approach was therefore to generate cultured cell populations from human airway tissue which naturally express the β_2 adrenoceptor, where gene expression is regulated by the same controlling elements as those present in vivo. These cell lines were genotyped to identify lines which were homozygous for the different polymorphisms and were then studied in greater detail. The third approach has been to study the airway responses of patient groups with respect to genotype.

The initial studies concentrated on amino acid 16 which can either be arginine (Arg) or glycine (Gly) depending on whether base 46 is an A or a G. A preliminary clinical study suggested that the Gly 16 form of the receptor might be associated with more severe asthma.[1] Asthma severity was judged mainly on clinical grounds in this study and consequently doubts were expressed regarding the clinical relevance of this polymorphism. However, more recent work has suggested that the ability of the receptor to desensitise is markedly influenced by the presence of a glycine at position 16. The Gly 16 form of the receptor downregulates following exposure to an agonist to a much greater extent than the Arg 16 form of the receptor in both transfected cell systems[12] and in primary cultured human airway smooth muscle cells.[13] Two recent clinical studies have supported the possibility that the Gly 16 form of the receptor is associated with markers of more severe asthma. Preliminary data from Dutch families with asthma suggest that Gly 16 may be associated with airway hyperreactivity.[14] In addition, patients with significant nocturnal worsening of their asthma were more likely to have the Gly 16 form of the receptor than asthmatic patients without nocturnal falls in peak flow rate.[15] A plausible explanation for this observation would be that individuals with asthma who have the Gly 16 form of the β_2 adrenoceptor and who use β_2 agonists during the day would be expected to downregulate their β_2 adrenoceptors such that they may then be susceptible to nocturnal worsening of their asthma. In support of this hypothesis, downregulation of β_2 adrenoceptors was seen in circulating white cells in the group with nocturnal asthma.[16]

The second polymorphism which has been studied in detail is at codon 27 which exists as either a glutamine (Gln) or as a glutamate (Glu) depending on whether base 76 is a C or a G. In contrast to the Gly 16 form of the receptor, the Glu 27 form of the receptor appears to protect against downregulation in both transfected and non-transfected cells systems.[12 13] Using primary cultured human airway smooth muscle cells we found that, following prolonged exposure to β_2 agonists, the Glu 27 form of the receptor downregulated to a much lesser extent than the Gln 27 form as assessed by changes in receptor number.[13] In addition, a similar relative resistance to downregulation was observed using β_2 agonist mediated cyclic AMP formation as a more functionally relevant end point for receptor coupling.[13] On the basis that individuals with the Glu 27 form of the β_2 adrenoceptor might be protected from bronchoconstrictor influences, we recently studied airway reactivity in a group of 65 mild to moderate asthmatics and found that individuals with the Glu 27 form of the receptor had four times less reactive airways than those with the Gln 27 form of the receptor when assessed using methacholine challenge. In this study heterozygotes had an intermediate mean PD_{20} value.[17]

The third polymorphism that has been studied is at amino acid 164 which can either be a threonine (Thr) or an isoleucine (Ile). This polymorphism is much rarer than the polymorphisms at amino acid 16 or 27 with an allelic frequency of about 1%,[1] but is potentially interesting in that amino acid 164 is situated in the fourth transmembrane spanning domain of the receptor and is adjacent to a serine at position 165 which has been predicted to interact with the β-carbon hydroxyl group of adrenergic ligands.[18 19] This polymorphism has been studied in a transfected cell system and has been shown to alter the agonist binding properties of the receptor.[19] Cells expressing Ile 164 were found to have approximately four times less affinity for ligands containing this hydroxyl group, whereas the binding of ligands such as dobutamine which lack this hydroxyl group were unaltered.[18] This alteration in binding affinity was reflected in a reduced capacity for the receptor to activate adenylyl cyclase relative to the wild type (Thr 164) form of the receptor. However, the rarity of this polymorphism has precluded clinical studies from being performed to date.

In addition to the above polymorphisms, a Japanese group has described a restriction fragment length polymorphism (RFLP) in the region of the β_2 adrenoceptor and has suggested that this RFLP may also be associated with asthma severity.[20] The RFLP is due to a Ban-I site which, if present, gives a 2.1 kb fragment in contrast with the 2.3 kb fragment seen otherwise following Ban-I digestion of the appropriate segment of DNA. Individuals with the 2.1 kb allele had lower responses to inhaled salbutamol than those with the 2.3 kb allele. The exact relationship of this RFLP to the polymorphisms discussed above remains to be defined because the position of the Ban-I site which is responsible for the RFLP has not been ascertained. It is even possible that this site may be outside the coding region of the gene.

Given the above associations between β_2 adrenoceptor polymorphisms and markers of asthma severity, is it possible that these polymorphisms may actually contribute to the asthma phenotype itself? The first important point to consider is that each individual has two genes for the β_2 adrenoceptor. Hence any individual can be either homozygous or heterozygous for a given polymorphism. The polymorphisms at position 16 and 27 are relatively common (the allelic frequency for Arg 16 in the sample of over 700 people we have assessed so far in Nottingham is 35% and for Gly 16 is 65%, whilst the allelic frequencies for Gln 27 and Glu 27 are 55% and 45%, respectively (unpublished data). The effects of these polymorphisms have generally been studied in homozygous cell lines or individuals, so the significance of polymorphisms in heterozygous individuals is unclear. Given that most individuals will be heterozygous, large populations will need to be studied in order to define the possible contribution of these polymorphisms to the asthma phenotype. Nevertheless, the observation that Glu 27 is associated with less reactive airways in asthmatic patients and that Gly 16 is associated with bronchial hyperreactivity implies that these polymorphisms may be relevant to the asthma phenotype. The situation is further complicated by the observation that the Arg-Gly 16 and Gln-Glu 27 polymorphisms may be in linkage disequilibrium – that is, the two polymorphisms are not independently distributed in the general population. Because of these difficulties large population studies will

be required to define the importance of these polymorphisms in determining airway reactivity and the asthma phenotype. These studies are currently being performed.

If β_2 adrenoceptor polymorphisms are important in defining the asthma phenotype, one would expect linkage between markers for asthma and the region on chromosome 5 coding for the β_2 adrenoceptor gene. Interestingly, there is evidence for linkage to this region in recent studies,[21] although linkage has also been described to a number of other potential loci including the nearby cytokine gene cluster on chromosome 5[22] and the high affinity IgE receptor on chromosome 11.[23 24] Realistically, it would seem extremely unlikely that an abnormality in the β_2 adrenoceptor could explain all of the features of the asthma phenotype, although it would be reasonable to hypothesise that polymorphisms in this receptor might account for some of the propensity for an individual to develop asthma upon exposure to the appropriate environmental stimuli, given the presence of other contributing genetically determined factors. In addition, it seems likely that these polymorphisms may contribute to determining disease severity in affected individuals.

Finally, what about the future? There are two important research areas to follow up resulting from these initial studies. Firstly, the possibility that β_2 adrenoceptor polymorphisms may contribute to other airway diseases – for example, reversibility in chronic obstructive airways disease (COPD) – requires study. Secondly, the possiblity that these polymorphisms may alter the response to treatment with β_2 agonists requires further work. There is also a wider message from these studies in that it is likely that other receptor genes in the airways may exhibit similar polymorphisms which may account for some of the variability between responses in individuals. With this in mind recent developments in polymerase chain reaction-based screening for gene polymorphisms are likely to provide a wealth of data in both airway and non-airway research in the future.

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