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Tolerance with β_2-adrenoceptor agonists: time for reappraisal

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1 In spite of the widespread use of β_2-adrenoceptor agonists in the treatment of asthma controversy continues regarding their possible role in increasing asthma mortality and morbidity. There is however no evidence available to suggest that tolerance to the bronchodilator or anti-bronchoconstrictor effects of these drugs is responsible for the deleterious effects reported with the regular use of bronchodilators.
2 There is no conclusive evidence to suggest that tolerance develops to the bronchodilator effects of short-acting β_2-adrenoceptor agonists. Tolerance does however appear to develop to the anti-bronchoconstrictor effects of these drugs.
3 With regard to the long-acting β_2-adrenoceptor agonists, there is evidence to suggest that tolerance develops both to their anti-bronchoconstrictor, and bronchodilator effects. Tolerance was however demonstrated in the presence of improved symptom control, therefore the clinical relevance of this phenomenon is uncertain.
4 Systemic corticosteroids can modulate lymphocyte β_2-adrenoceptor function both preventing, and reversing tolerance. The situation regarding the effects of systemic or inhaled corticosteroids on modulating bronchodilator responses in asthmatics is less clear. There is some evidence to suggest that inhaled corticosteroids are unable to prevent bronchodilator or systemic tolerance to long-acting β_2-adrenoceptor agonists.
5 On the basis of the current evidence, the British Thoracic Society guidelines for the management of asthma appear appropriate with regard to their recommendations for the use of long-acting β_2-adrenoceptor agonists.

Keywords β_2-adrenoceptor tolerance subsensitivity asthma salbutamol salmeterol formoterol

Introduction

β_2-adrenoceptor agonists have played a key role in the treatment of asthma for some 30 years, being used both for the relief and prophylaxis of symptoms. Although their use is widespread, controversy continues regarding the role played by these agents in terms of the observed increases in asthma mortality, and deterioration in disease control apparently associated with their regular use. The more recent introduction of long acting β_2-adrenoceptor agonists has further fuelled the debate, particularly with regard to the issue of β_2-adrenoceptor tolerance which may theoretically occur with continuous use.

In this review the primary aim is to reappraise current thinking regarding the question of tolerance, and to assess its possible clinical relevance. The issue of safety and increased mortality in relation to the use of β_2-adrenoceptor agonists has been extensively reviewed elsewhere and will only be referred to here in the context of tolerance [1–7]. The facilitatory effects of disease modifying agents such as corticosteroids on β_2-receptors and tachyphylaxis will also be discussed, as this has implications in clinical situations where the two therapies are often used concurrently. Finally, we will consider whether the present state of knowledge requires an evolution in the current asthma management guidelines in terms of the rational use of β_2-adrenoceptor agonists.

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The mechanisms and investigation of tolerance

Prolonged receptor stimulation in many physiological systems results in a reduction in response to a given stimulus, i.e. tolerance develops. In the context of β_2-adrenoceptors there are two major patterns for the development of tolerance. Homologous desensitisation refers to the situation in which tolerance only occurs to the agonist which produced the desensitisation, whilst in heterologous desensitisation responses to other classes of agonist are also blunted. Homologous desensitisation is the more rapid process and involves a rapid uncoupling of the receptor from adenylate cyclase, internalisation of the receptor from the cell surface, cyclic AMP independent phosphorylation of the receptor, and a slower down-regulation of the receptor, characterised by a loss of total receptor binding sites from the cell. Heterologous desensitisation may involve receptor phosphorylation by a cyclic-AMP dependent protein kinase (a negative feedback mechanism) [8].

Tolerance to systemic β_2-mediated effects, and to airway β_2-effects in normal subjects is well recognised [9, 10]. It was therefore reasonable to expect that similar changes may be observed in the airways of asthmatics, and thus provide an explanation for the reported adverse effects of these agents.

When reviewing the results of studies investigating the issue of tolerance it is important to appreciate the methodology likely to be required to demonstrate such tolerance. It is necessary to distinguish between effects on resting bronchomotor tone (i.e. bronchodilator activity) and antibronchoconstrictor properties (i.e. functional antagonism) of these drugs, as tolerance may develop preferentially to one property rather than another. With regard to the bronchodilator properties, it is relevant to consider both the peak and the duration of a bronchodilator response. It is more likely that tolerance, a consequence of receptor down-regulation, will be demonstrated in conditions where a high level of receptor occupancy would normally be achieved. It may therefore be necessary to construct cumulative dose-response curves to determine maximum response following treatment with the drug, and to compare this with the maximum response obtained following placebo treatment. Before commencing a study to investigate the occurrence of tolerance it is obviously necessary to ensure, as far as possible, that tolerance may not already have developed as a result of previous exposure to β_2-adrenoceptor agonists. A run-in period, during which β_2-adrenoceptor agonists are excluded from treatment should therefore be included prior to the study periods. The population of asthmatics selected for study may also influence the outcome. Disease modifying agents such as corticosteroids may influence the development of tolerance as will be discussed below. Populations which are homogeneous with regard to their use of such drugs should therefore be selected. Unfortunately some studies do not conform with these criteria, and their results may therefore need to be interpreted with a degree of caution.

Short-acting β_2-adrenoceptor agonists

Bronchodilator tolerance

The evidence to date regarding the development of bronchodilator tolerance following the use of short acting β_2-adrenoceptor agonists is inconclusive. Repsher *et al.* [11] found that tolerance to the duration, but not the peak bronchodilator response to a single dose of salbutamol (170 μg) developed after 91 days treatment with salbutamol, 170 μg four times daily when compared with the response prior to treatment, but without placebo control. Weber and coworkers were able to demonstrate tolerance to both the peak and duration of the bronchodilator response to cumulative doses of terbutaline in a group of 13 asthmatics, following 12 weeks treatment with inhaled terbutaline, 500 μg four times daily. Eight of this group were receiving corticosteroid therapy, and in this small sample this did appear to protect against the development of tolerance [12]. In contrast, several other studies assessing treatment with both oral and inhaled β_2-adrenoceptor agonists by means of bronchodilator responses to single and cumulative doses of inhaled, intravenous, and subcutaneous β_2-adrenoceptor agonists have failed to show evidence of tolerance [13–15].

Whilst these studies examined the effects of conventional doses of β_2-adrenoceptor agonists it could be predicted that higher doses producing greater receptor occupancy may be likely to produce tolerance, on the other hand studies have demonstrated that, at least when given acutely higher than normal doses of β_2-adrenoceptor agonists produced additional benefit in terms of bronchodilatation, without significant additional side effects [16]. High dose therapy using both inhalers and nebulisers has been examined in this regard. Following a 2 week washout of β_2-adrenoceptor agonists, Lipworth *et al.* [17] compared systemic and bronchodilator responses to cumulative doses of inhaled terbutaline following 4 weeks treatment with conventional doses of terbutaline (500 μg, four times daily via a spacer) or high dose treatment (2000 μg, four times daily via a spacer) in 11 patients with chronic obstructive airways disease. Whilst systemic responses were blunted to a significantly greater degree following high dose treatment compared with low dose, there were no differences in the bronchodilator responses. Teale and co-workers [18] studied the bronchodilator responses of a group of 10 patients with severe obstructive airways disease both before and during 3 months treatment with nebulised terbutaline, 5 mg four times daily. Baseline PEFR and FVC improved during the treatment period, and there was no reduction in peak bronchodilator response to cumulative doses of terbutaline. This study did not however include a washout period, and was not placebo controlled.

Antibronchoconstrictor tolerance

With regard to the antibronchoconstrictor effects of these drugs, concerns have been raised in two main

areas. Firstly, does tolerance develop to these properties with chronic use? Secondly, does rebound hyperreactivity occur after cessation of regular therapy? When considering these issues it is important to distinguish between the various stimuli used to provoke bronchoconstriction. Whilst methacholine and histamine are predominantly direct smooth muscle stimulants, allergen challenge and AMP act indirectly by stimulating mast cells, with mediator release subsequently producing bronchospasm. The mechanism by which exercise induces bronchoconstriction in susceptible individuals remains unclear but it may involve changes in the osmolality of the pulmonary extracellular fluid [19]. There may therefore be differential effects of β_2-adrenoceptor agonists on these varied stimuli, with a consequent difference in susceptibility to tolerance.

Cockcroft *et al.* [20] studied the response of 11 mild asthmatics to allergen and methacholine challenge. Responses were measured before, and after 2 weeks treatment with inhaled salbutamol, in a dose of 200 μg four times daily or placebo, with and without administration of salbutamol immediately prior to the provocation test. Having withheld salbutamol they showed no change in the response to methacholine challenge whilst there was an increase in sensitivity to allergen, equivalent to 0.91 doubling doses, following regular treatment with salbutamol compared with placebo. Furthermore, there was a reduction in the acute protective effect of salbutamol against both allergen and methacholine challenge following regular treatment with salbutamol, although the effect was more marked with allergen challenge. It was speculated that this may have been a result of β_2-receptor down-regulation allowing enhanced mast cell mediator release by reducing the ability of the agonists to stabilise the mast cell, a β_2-receptor mediated process. In this regard it has also been shown, in 12 mild asthmatics, following 7 days treatment with terbutaline, that tolerance developed to the protective effect of terbutaline against AMP induced bronchospasm. The reduction in protection was of the order of 2.1 doubling doses [21]. As in the study by Cockcroft a reduction was seen in the protection against methacholine challenge. However, both the initial level of protection and the subsequent reduction were less than was the case for AMP. Results using histamine as the stimulus have been less consistent, with some groups demonstrating a statistically significant increase in reactivity following regular treatment with β_2-adrenoceptor agonists whilst others have not [15, 22–25]. In the case of exercise induced bronchospasm a reduction in the protective effect of salbutamol has been demonstrated in six mild asthmatics after between 4 and 20 weeks treatment with oral salbutamol. This effect was not however seen in six adolescents receiving inhaled salbutamol [26].

All these results must be interpreted in the light of an awareness of the intrinsic variability of bronchial hyperreactivity. Even in an ostensibly stable clinical situation, measurements of reactivity can vary by up to two-fold, i.e. +/− one doubling dose [27]. It can therefore be seen that whilst statistically significant, some of the findings discussed may be of more doubtful clinical significance. It may be that although as a whole the changes seen are relatively small when compared with normal biological variability, in a subgroup of more severe asthmatics such changes may assume relatively more importance. It is, however, worth noting that studies quoted have used relatively mild asthmatics not receiving disease modifying therapy, and the results should not automatically be extrapolated to more severe cases.

Whilst investigating the question of tolerance to antibronchoconstrictor effects it was observed in some studies, that bronchial reactivity appeared to increase in the period immediately after stopping regular β-adenoceptor agonist therapy [23]. Vathenen *et al.* [28] measured bronchial reactivity to histamine for up to 24 h after completing 2 weeks treatment with terbutaline. As in other studies, they demonstrated a significant reduction in the protection afforded by a dose of terbutaline against histamine induced bronchoconstriction following this treatment. They also demonstrated an increase in bronchial hyperreactivity after stopping treatment with a maximum increase of 1.5 doubling doses at 23 h. Larsson and co-workers [29] demonstrated a trend towards increased reactivity to allergen challenge 48 h after stopping treatment with oral terbutaline. Down-regulation of β_2-receptors has been invoked as an explanation for this phenomenon, as this may render the subject less able to respond to endogenous catecholamines. The relevance of these relatively small changes to disease control remains unclear, and in any event the sudden cessation of regular therapy is a relatively unlikely clinical scenario.

In summary, there is little evidence to suggest that significant tolerance develops to the bronchodilator properties of short-acting β_2-adrenoceptor agonists. More evidence has accumulated with regard to tolerance to antibronchoconstrictor effects. This is not however, a uniform phenomenon, with more evidence relating to the acute protection afforded against agents causing mast cell degranulation, where β_2-adrenoceptor agonists have a direct, receptor mediated role in stabilising the cells.

Disease control—is tolerance relevant?

Is there any evidence to support the hypothesis that detrimental effects of regular β_2-adrenoceptor agonist therapy may be due to the development of tolerance to bronchodilator effects or because of decreased protection against bronchoconstrictor stimuli? In this respect three published studies have compared regular *vs* as required treatment with β_2-adrenoceptor agonists. Sears *et al.* [30] claimed that treatment with high dose inhaled fenoterol (400 μg four times daily) compared with β_2-adrenoceptor agonist given as required resulted in a deterioration in a number of parameters of disease control, although increased bronchial hyperreactivity was only reported in 34% of cases. It is difficult to assess whether tolerance was responsible for the observed effects as proper dose-response curves were not constructed. Subgroup

analysis revealed that deterioration occurred equally in those receiving β_2-adrenoceptor agonist monotherapy compared with those receiving co-therapy with inhaled corticosteroids, suggesting that there was no facilitatory effect of corticosteroid in this situation. The study of van Shayck *et al.* [31] where regular treatment with ipratropium bromide or salbutamol was associated with a greater decline in FEV_1 compared with on demand treatment (mean difference of 52 ml per year) is difficult to interpret, primarily because bronchial hyperreactivity, symptom control, and quality of life were unaltered whilst data on bronchodilator dose-response curves were not reported. Finally, more recently, Chapman *et al.* [32] reported that regular salbutamol improved symptoms compared with as required treatment although parameters of disease activity were not evaluated as such.

It therefore appears that if regular β_2-adrenoceptor agonist therapy has a deleterious effect on lung function or disease control, the effect is of small magnitude, and perhaps not of clinical relevance. The question as to whether tolerance is responsible for altered disease control remains unanswered on the basis of the available studies.

Long-acting β_2-adrenoceptor agonists

Salmeterol and formoterol are both β_2-adrenoceptor agonists with a duration of bronchodilator action of at least 12 h [33, 34]. Salmeterol is a partial agonist at the airway β_2-receptor *in vitro*, whilst formoterol is a full agonist in this situation [35]. Whilst such a prolonged duration of bronchodilatation may be con-sidered beneficial, particularly with regard to nocturnal symptoms of asthma, it may be anticipated that the prolonged receptor occupancy produced by this newer group of β_2-adrenoceptor agonists would be more likely to result in receptor down-regulation, and therefore result in tolerance, than is the case with the shorter acting drugs. Furthermore, the distinction between the full agonist activity of formoterol, and the partial agonism of salmeterol may be relevant in this regard. In the light of these theoretical concerns several studies have been carried out to evaluate the possible occurrence of tolerance to both the bronchodilator and antibronchoconstrictor effects of these agents.

Bronchodilator tolerance

Ullman *et al.* [36] treated 12 asthmatics (11 of whom were taking inhaled corticosteroids) with salmeterol 50 μg twice daily or salbutamol 200 μg four times daily for 2 weeks, but without a placebo control period. At the end of each treatment period dose-response curves to salbutamol were constructed, using a total cumulative dose of 900 μg. During the treatment period asthma control was said to be significantly better with salmeterol compared with salbutamol, in terms of peak flow measurements, sleep quality and degree of dyspnoea. This study also concluded there was no evidence of tolerance to the bronchodilator dose-response to salbutamol, although the methodology employed may have masked this, particularly as they were unable to reproduce the well documented phenomenon of systemic tolerance following chronic dosing with salbutamol. The results may therefore reflect the absence of a run-in period without β_2-adrenoceptor agonists prior to the study such that tolerance could have been present from the outset. Furthermore, the basal values of forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were significantly increased after treatment with salmeterol compared with pretreatment values. This makes comparison between dose-response curves difficult, since whilst the absolute final values of FEV_1 and FVC were similar, the change from baseline was less after treatment with salmeterol. It was not possible to elucidate whether this reflects the fact that the patients had reached a ceiling in the dose-response curve or whether this was indeed a manifestation of tolerance. Other studies have concurred in finding no evidence of tolerance to the bronchodilator effects of salmeterol although the findings were based on responses to single doses of salmeterol, and in the absence of a run-in period prior to the study [37, 38].

Arvidsson and co-workers [39] evaluated formoterol in 20 asthmatics (15 of whom were using inhaled corticosteroids) with regard to symptom control and tolerance. Patients received formoterol, 12 μg twice daily, in a crossover study with salbutamol, 200 μg twice daily, each for 2 weeks, again without placebo control or run-in period. Bronchodilator responses to a total cumulative dose of 1300 μg salbutamol were assessed at the end of each treatment period. The baseline value of FEV_1 was increased after treatment with formoterol compared with pretreatment values, presumably because of its long duration of action. Thus comparisons of the responses to salbutamol are difficult to evaluate properly. The authors concluded that as the maximum FEV_1 achieved was similar before, and after treatment with formoterol, bronchodilator tolerance did not occur. However the same argument clearly applies as with the study of Ullman *et al.* [36] regarding a ceiling in response being achieved. In two placebo controlled studies, one with a metered dose inhaler, and the other with a dry powder device, both employing a 2 week run-in period (during which the anticholinergic ipratropium bromide was substituted for rescue use instead of β_2-adrenoceptor agonists), and with dose-response curves constructed with formoterol (up to 126 μg cumulative dose), we have found evidence of tolerance to the bronchodilator effects of formoterol, after 4 weeks treatment with formoterol at a dose of 24 μg twice daily [40, 41]. This was manifest as a significant reduction in the peak bronchodilator response, together with an even greater reduction in the response measured 6 h after the final dose of the dose-response curve. Indeed these effects were obtained for FEV_1 and FEF_{25-75}, perhaps suggesting that subsensitivity occurred for both small and large airway β_2-receptors (Figure 1). In these studies the

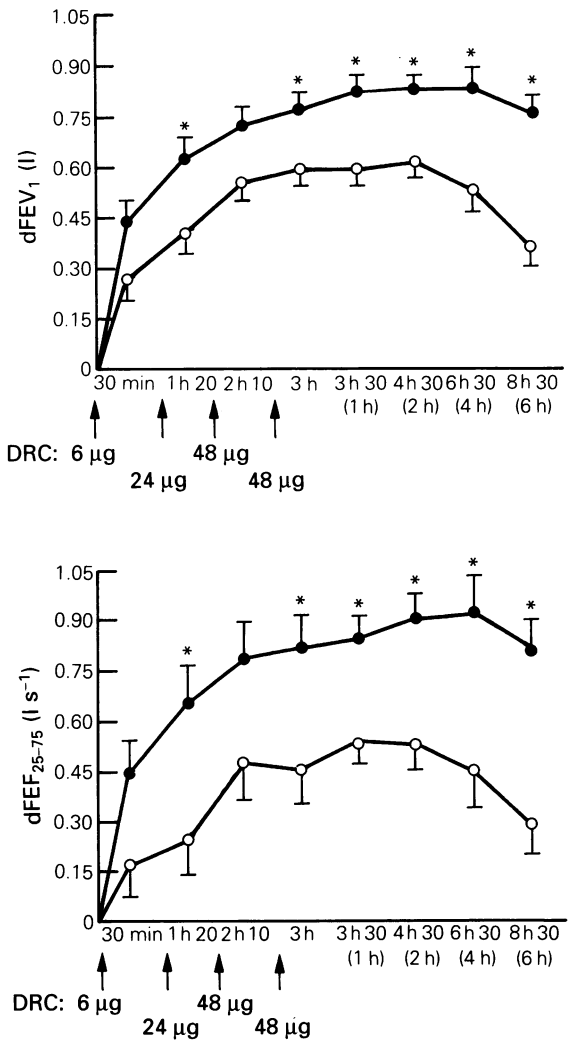

Figure 1 Response time profile for FEV_1 and FEF_{25-75} shown as delta (d) from baseline, after treatment for 4 weeks with either placebo (●) or formoterol 24 µg twice daily (○). Measurements were made over a 20 min period beginning 30 min after each dose with increments during the dose-response curve made every 50 min. Times are given following the first dose of formoterol (6 µg), and in brackets for time after inhalation of the last dose (48 µg). Asterisks denote a significant difference between the value after treatment with placebo compared with formoterol. Values are shown as means and s.e. mean. (Taken from reference 40, with kind permission of Yorke Medical Journals, New York, USA).

fact that there were no significant differences between the baseline measurements makes comparisons of the dose-response curves valid in terms of comparisons of delta-responses. Predictably formoterol also produced tolerance of extra-pulmonary β_2-responses compared with placebo. Interestingly peak flow measurements were markedly improved by formoterol which might conceivably result in patients being lulled into a false sense of security in that their airway β_2-receptors were clearly subsensitised. It may also be relevant that in both studies most patients were receiving inhaled corticosteroids suggesting that the latter do not protect against β_2-receptor subsensitisation in airways.

Interestingly, in the same studies [40, 41], bronchodilator tolerance was associated with *in vitro* evidence of β_2-receptor down-regulation, as assessed by lymphocyte parameters of receptor binding density and cyclic AMP response to isoprenaline. In theory therefore, this may provide an accessible tool for assessing *in vivo* β_2-receptor function. Controversy, however exists as to whether it is valid to extrapolate from changes seen in circulating lymphocytes *in vitro* to changes in pulmonary receptors. Tashkin *et al.* [15] demonstrated lymphocyte β_2-receptor down-regulation in asthmatics following treatment with oral terbutaline whilst bronchodilator responses to inhaled isoprenaline and subcutaneous terbutaline were maintained [15]. Hauck and co-workers examined the relationship between lymphocyte and pulmonary β_2-receptors directly [43]. Eighteen patients undergoing lung resection were evaluated. Ten received subcutaneous terbutaline 0.5 mg twice daily for 24–72 h preoperatively whilst eight received no treatment. It was found that whilst mononuclear leucocyte β_2-receptor density was reduced by treatment, there was no significant change in lung receptor density. Whilst this could represent a constitutive difference in susceptibility of the two receptor populations to down-regulation it is also possible that inadequate tissue penetration was achieved with the dose and route of administration used, or that the pulmonary receptors were already down-regulated as a result of previous exposure to β_2-adrenoceptor agonists, as was the case in half of the treatment group. Thus the validity of this technique as an index of pulmonary β_2-receptor function remains unclear.

However, recently abstracted *in vitro* data has indeed suggested that lung β_2-receptors become down-regulated on exposure to long-acting, but not short-acting β_2-adrenoceptor agonists [44]. Interestingly it was also found that salmeterol increased the receptor association constant, a finding mirrored in our two studies with formoterol [40, 41]. This perhaps infers that increased receptor affinity may in some way be compensating for the reduction in receptor density. Quing *et al.* [45] have also recently reported, in abstract form, that in normal subjects both lung (as assessed by positron emission tomography) and mononuclear leucocyte β_2-receptors undergo down-regulation following exposure to β_2-adrenoceptor agonists, and concluded that lymphocyte β_2-receptor density may be used as a surrogate for following airway β_2-effects [45].

Antibronchoconstrictor tolerance

Following acute dosing, salmeterol and formoterol produce prolonged protection against a variety of bronchoconstrictor stimuli [46–48]. Studies have been performed to elucidate whether tolerance develops to this antibronchoconstrictor property. Cheung and co-workers studied 24 patients with mild asthma requiring treatment with salbutamol on an as required basis only [37]. They received 8 weeks treatment with either salmeterol 50 µg twice daily or placebo. The protective effect of salmeterol against metha-

choline induced bronchoconstriction was studied after the first dose, and again after 4 and 8 weeks of treatment. The challenges were performed 1 h after receiving a dose of salmeterol but this was preceded by a 36 h period during which medication was withheld. The results showed that the acute protective effect of salmeterol against histamine was markedly attenuated after 4 weeks of treatment. In this study salmeterol was given to a group of very mild asthmatics, not requiring disease modifying therapy, i.e. a group of patients who would not normally receive salmeterol as routine therapy. It may therefore not be valid to extrapolate these findings to more severe asthmatics. It is probably more meaningful to consider studies where the protective effect of salmeterol was evaluated within the normal dosing interval (i.e. at 12 h). Booth and co-workers studied a population of mild to moderate asthmatics, the majority of whom (73%) were receiving treatment with inhaled corticosteroids [49]. Subjects received 8 weeks treatment with placebo or salmeterol, 50 μg twice daily, and methacholine challenge tests were performed 12 h after the first dose, at 4 and 8 weeks of treatment, and during a subsequent washout period. In this group of patients the protective effect of salmeterol seen after the first dose (0.6–1.2 doubling doses) was not significantly altered after chronic dosing. Beach *et al.* [50] studied 20 patients, all of whom were receiving inhaled corticosteroids. Bronchial reactivity to methacholine was measured 24 and 72 h after ceasing 6 weeks of continuous treatment with salmeterol, 50 μg twice daily or salbutamol 400 μg twice daily, but without placebo control. There were no significant changes in bronchial hyperreactivity within or between the two treatment groups.

There is, however, evidence to show that tolerance develops to the protective effect of salmeterol against exercise induced bronchoconstriction, in a study where salmeterol 50 μg twice daily was compared with placebo given for 4 weeks, with exercise challenge performed 6 and 12 h after the first dose and at 4 weeks. Whilst salmeterol protected against exercise induced bronchoconstriction compared with placebo at 6 and 12 h after the first dose, there was no significant difference between salmeterol and placebo after 4 weeks of treatment [51] (Figure 2). It is worth pointing out that the patients studied had only mild asthma, and only three patients were using inhaled corticosteroids. Recent data has compared the effect of salmeterol 50 μg twice daily for 4 weeks with placebo, in 17 asthmatics, all receiving inhaled corticosteroids, after an initial 2 week run-in without β_2-adrenoceptor agonists. Whilst morning peak flows were improved during salmeterol treatment, histamine challenge performed 12 h after the last dose of each treatment period showed only minimal protection (i.e. 0.7 doubling doses) with salmeterol compared with placebo. Furthermore a bronchodilator dose-response curve to salbutamol performed 36 h after the last dose showed evidence of a parallel right shift after salmeterol compared with placebo with a four-fold greater dose of salbutamol being required to produce the same PEFR response [52]. Lymphocyte β_2-receptor density showed a trend towards down-regulation after treatment with salmeterol, with unchanged receptor affinity, compared with placebo or run-in. Thus it appears that salmeterol, like formoterol, produces bronchodilator tolerance which is mirrored by β_2-receptor down-regulation.

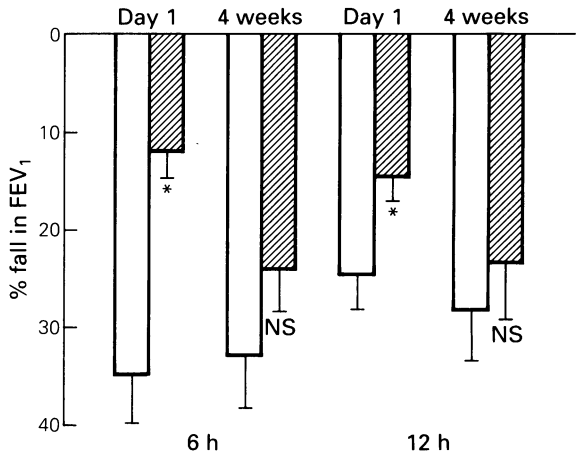

Figure 2 Percentage fall in FEV_1 after exercise challenge performed at 6 h and 12 h after the first dose (day 1) and after 4 weeks of continuous inhaled salmeterol 50 μg twice daily (▨) or placebo (□). Values are means and s.e. mean. Asterisks denote a significant difference between salmeterol and placebo. (Taken from reference 51, with kind permission of W.B. Saunders Ltd, London, UK).

Data are not yet available regarding chronic dosing with formoterol in these situations. It may also be interesting to study the protective effect of chronic dosing with salmeterol or formoterol on bronchial reactivity to AMP, as experience with short-acting agonists suggests that this response may be more susceptible to tolerance, possibly because of the receptor mediated process involved in producing bronchospasm in this case.

The influence of disease modifying therapy

The use of disease modifying therapy is now advocated at an early stage in the management of asthma [53]. The influence of such therapy on the action of β_2-adrenoceptor agonists is therefore of great clinical relevance.

Corticosteroids are thought to be heterologous regulators of β-adrenoceptors in many tissues [54]. This includes considerable evidence to suggest that *in vitro*, corticosteroids modulate β_2-receptor function on circulating lymphocytes. Hui *et al.* [55] demonstrated that a single dose of methylprednisolone restored β_2-receptor density to normal values within 16 h, the receptors having previously been down-regulated by oral terbutaline therapy. The steroid had no effect on receptors that had not been down-regulated. A single dose of 100 mg oral prednisolone produced similar up-regulation, within 8 to 10 h of receiving

the drug [56]. The mechanism of this effect is not yet fully understood, but it may involve an increase in the rate of *de novo* synthesis of receptors or, a reversal or inhibition of internalisation of receptors from the cell surface. The human β_2-adrenoceptor gene has at least three glucocorticoid response elements (GREs) in its promoter region. Activated glucocorticoid receptor-steroid complexes bind to these sites and increase gene transcription. Corticosteroids are also thought to promote the formation of the coupled, high affinity state of the receptor, which will in turn increase receptor function [54]. β_2-adrenoceptor agonists are also thought to be able to influence the binding of corticosteroids to DNA. β-adrenoceptor agonists can increase binding to cAMP response elements in human lung *in vitro*, and at the same time reduce binding to GREs [57]. In theory, this could suggest that in high concentrations β-adrenoceptor agonists may inhibit the anti-inflammatory actions of steroids.

Does up-regulation of β_2-receptors *in vitro* correlate with improved function of airway β_2-receptors? Holgate *et al.* [10] studied normal subjects, in whom it is possible to reliably produce tolerance to the bronchodilator effects of β_2-adrenoceptor agonists. Having produced tolerance, they found that full bronchodilator sensitivity was restored 3–5 h after an intravenous injection of 200 mg hydrocortisone. In the 1970s a small study of 10 asthmatics who were said to be 'refractory' to the bronchodilator effects of isoprenaline showed that a single dose of prednisolone restored bronchodilator responses within 1 h [58]. Brodde *et al.* [59] attempted to correlate changes in lymphocyte β_2-receptor density with changes in airway responses to inhaled salbutamol in a group of asthmatic patients. These patients with stable disease had lower than normal lymphocyte β_2-receptor density, and reduced cyclic AMP responses to isoprenaline, presumably as a consequence of regular β_2-adrenoceptor agonist therapy. These changes were reversed to normal 16 h after an intravenous injection of prednisolone, 100 mg. Changes in the lymphocyte β_2-receptor parameters were associated with an increase in prebronchodilator peak flows, but there was no change in the bronchodilator response to salbutamol. Although the numbers of patients involved have been small, studies which have demonstrated bronchodilator tolerance also suggest that using inhaled steroids may protect against tolerance [12].

There is now good evidence to suggest that when inhaled corticosteroid and β_2-adrenoceptor agonist are given concurrently bronchial hyperreactivity remains suppressed. In a multi-centre parallel group study of 274 patients followed up for up to 24 months, the combination of inhaled beclomethasone diproprionate (800 μg daily) and β_2-adrenoceptor agonist (terbutaline 2000 μg daily) was compared with β_2-adrenoceptor agonist alone, or anti-cholinergic alone (ipratropium bromide 160 μg daily) [60]. The results showed that FEV_1 increased by 10.3% of predicted normal, and bronchial reactivity to histamine decreased (by 2.0 doubling doses) only in the combined group, and was unchanged in the group receiving bronchodilator alone. Furthermore these effects were maintained over the 24 months of follow-up (Figure 3). In a smaller study of 27 asthmatic children, the combination of budesonide (400 μg daily) and terbutaline (2000 μg daily) produced a reduction in histamine reactivity with a 2.1 doubling dose difference compared with terbutaline plus placebo [25]. Finally Paggiaro *et al.* [61] compared the early and late response to allergen after 1 week of treatment with salbutamol (900 μg) daily plus beclomethasone dipropionate (600 μg daily), or salbutamol plus placebo. Both early and late responses were blunted after the combined regime but not with β_2-adrenoceptor agonist alone. These studies indicate it is probably safe to give regular β_2-adrenoceptor agonists providing patients are also taking inhaled steroid. However since requirements for regular β_2-adrenoceptor agonist is an indication of persistent disease activity it is probably more rational to

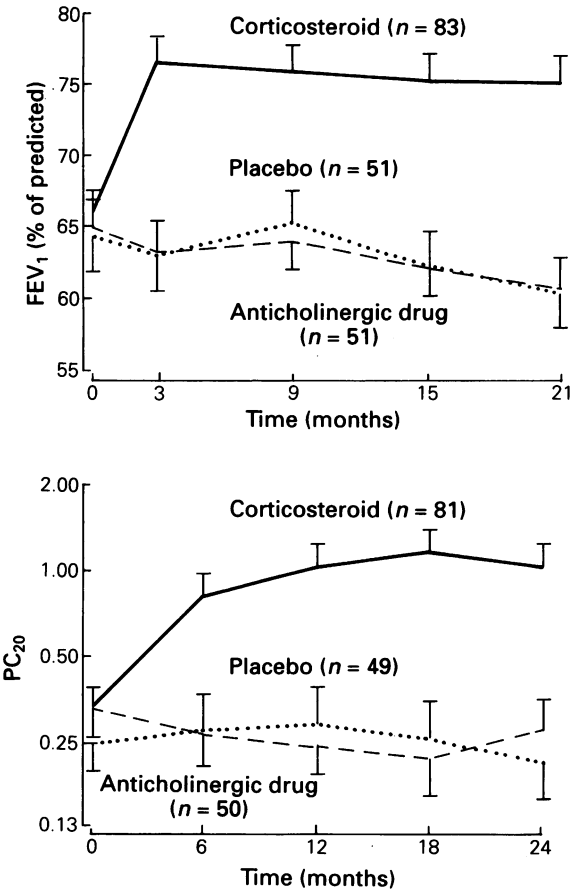

Figure 3 The effects of regular inhaled terbutaline 2000 μg daily in combination with either: beclomethasone dipropionate 800 μg daily ('corticosteroid'), ipratropium bromide 160 μg daily ('anticholinergic') or 'placebo' on a) FEV1 before bronchodilatation and b) PC_{20} for FEV_1 in response to histamine, in patients with obstructive airways disease. Time zero indicates the time of randomisation. (Taken from reference 60, with kind permission of the Massachusetts Medical Society, USA).

titrate the dose of inhaled steroid such that patients only require to use β_2-adrenoceptor agonists on demand, hence suppressing the underlying inflammatory cascade.

Whether the same protective effect of inhaled corticosteroids is seen in patients taking long-acting β_2-adrenoceptor agonists is unclear. Certainly bronchodilator tolerance has been demonstrated with formoterol in patients taking concurrent treatment with inhaled corticosteroids [40, 41]. With respect to the long-acting β_2-adrenoceptor agonists such as salmeterol the question has arisen as to whether it is best to optimise the dose of inhaled corticosteroid in the step-up phase of management, or to add salmeterol to a lower dose of inhaled corticosteroid. In a recent study by Greening and co-workers, in a group of 429 mild asthmatics with persistent symptoms whilst taking beclomethasone dipropionate 400 μg daily, the addition of salmeterol 50 μg twice daily was more effective in controlling symptoms than increasing the dose of steroid to 1000 μg daily. Over the 6 month study period there was no difference between treatments in terms of the exacerbation rate [62]. It is perhaps not surprising that in the presence of prolonged bronchodilatation both symptoms and peak expiratory flow were significantly improved in the salmeterol limb of the trial. This study received methodological criticism in that beclomethasone dipropionate was delivered by metered dose inhaler whilst the salmeterol was delivered by dry powder device [63]. Thus it is likely that the study was heavily biased against the steroid limb in view of the notoriously poor inhaler technique with metered dose inhalers.

Other disease modifying therapies have been shown to have an effect on β_2-receptor function. Ketotifen has been shown both to accelerate up-regulation of previously down-regulated lymphocyte β_2-receptors, and to attenuate down-regulation. This has been found to correlate with an attenuation of the tolerance to systemic, and airway β_2-mediated effects in normal subjects [56, 64]. Ketotifen has also been shown to up-regulate lymphocyte β_2-receptors in asthmatics, a change associated with an improvement in pre-bronchodilator peak flows but no change in reversibility [59]. The mechanism of this effect is not yet known but ketotifen may interfere with internalisation of β_2-receptors. The anti-inflammatory agent nedocromil sodium has also been shown to prevent isoprenaline induced β_2-receptor down-regulation of guinea-pig lung, *in vitro* [65]. *In vivo* studies using a suitable model of bronchodilator tolerance may therefore be informative in indicating novel applications for these compounds which, to date have had a limited role in the management of adult asthmatics.

Conclusions and the way forward

After numerous studies, no consistent picture of bronchodilator tolerance to short-acting β_2-adrenoceptor agonists has emerged, making a significant clinical problem unlikely. Although changes in bronchial hyperreactivity have been demonstrated more readily, many of the changes are small, making them of limited significance, except possibly in certain sub-groups of patients. In any event adverse effects on bronchial hyperreactivity are not observed in those patients taking concomitant therapy with inhaled corticosteroids.

Bronchodilator tolerance has been demonstrated with formoterol and with salmeterol. The results regarding tolerance to the antibronchoconstrictor properties of these agents have been conflicting, and complicated by varying methodology in terms of timing of the challenge. Results obtained in mild asthmatics should not be extrapolated to the more clinically relevant setting of more severe asthmatics requiring disease modifying therapy. More work is required in this area, and to study the effects of formoterol in this regard.

Although questions still remain regarding the issue of tolerance and long-acting β_2-adrenoceptor agonists, it should be noted that the use of these agents is generally associated with an improvement in symptom control. What then is the clinical significance of this phenomenon? It is possible that patients may be rendered less responsive to bronchodilator therapy administered in an acute exacerbation. It is also conceivable that in the presence of prolonged bronchodilation symptoms associated with inadequate control of the inflammatory process will be suppressed. In other words patients may in effect be lulled into a perceived false sense of security, particularly if peak flow measurements are maintained, as is often the case. Studies are clearly warranted to investigate this issue further. The acute effect of corticosteroids on bronchodilator responses in such a situation may also be of interest, as the *in vitro* data available suggests that corticosteroids can act very rapidly to modulate adrenoceptor function. Thus corticosteroids may be beneficial by their acute facilitatory effects on β_2-adrenoceptors, and not only by virtue of their anti-inflammatory properties, which have a longer time course of action.

Adequate disease modifying therapy is crucial to the successful management of asthma, in terms of suppressing the inflammatory cascade. It is therefore evident, from these principles that the long-acting β_2-adrenoceptor agonists should only be considered in the context of such combined therapy, once the patients have been titrated to an optimal dose of inhaled corticosteroid. Caution is warranted before advocating departure from the principles of producing adequate suppression of inflammation, in terms of adding long-acting β_2-adrenoceptor agonists to sub-optimal doses of inhaled corticosteroids. However in the context of combined therapy with optimal doses of corticosteroids there is no evidence at present to suggest that long-acting β_2-adrenoceptor agonists cannot be used safely, in keeping with the current guidelines.

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