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Asthma treatment through the beta receptor: lessons from animal models

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1. ABSTRACT

Asthma is a significant health problem worldwide with a prevalence that continues to rise and for which there is no cure. Animal models have been used for decades to investigate the cause and cures of asthma, and while they do not always mimic many of the facets of this syndrome, mechanistic animal studies are still nevertheless very useful. Animal studies with beta-agonists suggest much broader and perhaps more important roles for beta-agonists since beta-agonists reduce aspects of inflammation and may affect structural remodeling. Studies using enantiomers of beta-agonists provide a confusing picture of the degree and mechanism of the deleterious effects of racemic mixtures and/or the S-enantiomer or other classes of beta-agonists. Neural mechanisms are implicated. The future holds a promise of even more insight into the mechanisms of the acute and chronic role of the beta-adrenoceptor, asthma therapeutics, in particular, beta-agonists that will lead to a better understanding of the pathogenesis and treatment of asthma.

2. INTRODUCTION

2.1. Animal models and asthma

Animal models provide critical correlations between mechanisms uncovered at the bench and the *in vivo* context. Animal experiments allow unraveling of the mechanisms that cause human disease, in this case the syndrome of asthma. In addition, animal models provide essential proof-of-concept evidence for the effectiveness of potential therapeutics. *In vivo* animal experiments can also further test the hypotheses developed from clinical observations where a more complete and detailed picture of the process at work can be obtained. The role of animal models has been bidirectional and pivotal to our current understanding of the complexities of the pathogenesis of asthma. (Figure 1).

Prior to the end of the 1980s, a variety of animal species had been employed to assess potential therapeutics and mechanisms (1) and yet no real consensus arose from these extensive studies as to what model or what species

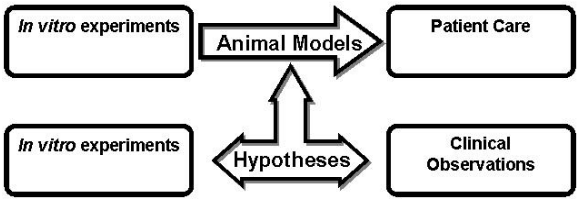

Figure 1. The role of animal models in asthma research. Animal models have served to develop *in vitro* experiments from the bench into *in vivo* experiments in animal models and then human therapeutic clinical trials ("proof-of-concept"). Conversely, animal models have been used to test mechanistic hypotheses based on human observations that require better controlled *in vivo* experiments and often, further study *in vitro*. These mechanistic studies are then used in the reverse direction for *in vitro* experiments and to develop clinical therapies.

was most informative. Since then, the utilization of the small laboratory animals, especially the mouse, as a model system has clearly eclipsed the use of all other species; moreover, the amount of information derived from these current investigations is truly remarkable. Nevertheless, other laboratory species may offer unique insights. Consequently, there are a number of reviews and opinion articles that espouse the value of other animal species and animal models in general to investigate asthma mechanisms (2-12).

What are we modeling with animal "asthma" models? Asthma is a clinical syndrome characterized by inflammation, reversible airflow obstruction, periodic airflow obstruction (e.g., nocturnal asthma) and airways hyperresponsiveness (13). More recently, the role of airway structural changes or remodeling has emerged as an important aspect of asthma pathogenesis (14). It is becoming increasingly clear that the exact nature of the cellular inflammation that occurs in the airways and parenchyma of the lung is complex and not easily characterized by a single or simple conceptual model. One problem is that the pathophysiological features of asthma are not specific. Reversible airflow obstruction is not a specific feature of asthma since this feature occurs in many other lung diseases. In like fashion, airways hyperresponsiveness (AHR) is also not specific to asthma as patients with other disorders, e.g., COPD or cystic fibrosis, also exhibit AHR (13). Periodic airflow limitation and AHR are two cardinal features of asthma that are often closely correlated to the inflammatory process (13,14). Yet it is only AHR that is most often used as an endpoint in studies of asthma pathogenesis in laboratory animals (2,10). As a result of this focus, we currently understand best the myriad of inflammatory factors and mechanisms that lead to AHR in like fashion. An effective asthma therapeutic would ameliorate asthmatic symptoms, airflow obstruction or AHR; a great asthma therapeutic would be effective in all domains of disease expression and furthermore lead to an improvement in asthma control. This later clinical condition will be a much more difficult situation to model.

2.2. Mechanisms of dysfunction in asthma

Traditionally, asthma was thought to be due to abnormal neural control resulting in excessive airway smooth muscle constriction and airway narrowing; in this paradigm beta-agonists are a rational therapy because of their ability to relax airway smooth muscle from a pre-contracted state. This view changed when it was shown that asthma symptoms were strongly correlated to serum IgE (15). Asthma is now known to be much more than abnormal smooth muscle contraction (16). The current view is that asthma and AHR occur due, in part, to a skewing of the adaptive immune response (10,13,14,17). This is characterized by a T-helper type 2 (TH2) cytokine such as IL-4, IL-5 and IL-13 and increases in CD4+ T-cells (17-20). Currently, even this hypothesis is too simplistic and may not be directly applicable to the human condition of asthma. Mounting evidence suggests that another population of lymphocytes, e.g., Treg or Th 17 cells, may be involved (18,19,21). Furthermore, asthma may also manifest a variety of mechanistic phenotypes (22,23) or, in other words, asthma can be caused by more than one mechanism, and animal models clearly demonstrate that principle (24). Animal models have shown that allergen can modulate smooth muscle function (24), increase epithelial permeability (25) or enhance mucous obstruction (26). However, how these changes may interact with or even which is most important remains unclear.

Animal models have provided invaluable tools defining the role of specific components in the rapidly expanding understanding of asthma pathophysiology. Examples include, but are not limited to, the role of the various cell types such as eosinophils (27-29), genetic variation in asthmatic phenotypes (27,30), differing phases in the development of allergic inflammation, the role of epithelium in orchestrating the response to antigen (31,32), and the complex effects of inflammation on airway muscle (32,33). Beta receptors are found in multiple locations in the airway (34) suggesting heterogeneous actions and roles that may depend on the location. Recent investigations in animal models suggest that the beta receptor and beta-agonists have a more multifaceted role in this complex process.

2.3. Acute antigen challenge models

No animal except perhaps the cat or the horse with heaves (1) has a naturally occurring syndrome akin to the asthma syndrome in humans. As with many other asthma models, the approach most widely used is to immunize (sensitize) animals to a protein or hapten and then challenge after a suitable period of time with the same antigen (2). While simple in concept, the practical details of any protocol are often confusing and highly variable (35). These facts make the comparison between results from different studies and laboratories fraught with problems and uncertainty.

Most animal models use an acute challenge protocol because of the reduced cost and expediency; however, such models have the disadvantage that animal antigen exposures are short whereas in the human situation, patients are often exposed to antigen for long periods of

time (years). Nevertheless, the acute models are still relevant to pediatric asthma where the exposures are over a shorter term (36). Hence, these acute exposure models remain relevant to mechanisms that initially cause or sustain the asthma syndrome. These acute systems may also replicate situations in milder asthmatics where there is a complete or near complete resolution of inflammation between bouts of asthma and inflammation.

2.4. Chronic animal models

Chronic exposure models seek to simulate the more chronic exposures that asthmatics undergo throughout their lives. However, it is nearly impossible to know what doses or schedule of exposure asthmatics are subjected to, and so it is no surprise that there is a great deal of variability in the protocols that are employed (12,37,38). The studies using chronic exposure systems are accordingly highly variable among laboratories (35) and the changes in AHR indeed are small in magnitude (that is less than a 1 log shift in responsiveness). Given the variability in the animal models, there is little consensus about important mechanisms that cause AHR or other features of the asthma syndrome.

2.5. Use of animal models to determine therapeutic effectiveness

Animal models have long been employed to explore the effectiveness of therapeutic agents. Such approaches are fraught with challenges such as the route of delivery and dosage of the investigational drug. Positive outcomes, however, indicate a potential new therapeutic action and support for a particular mechanism if the drug is highly selective in its action (10). Accordingly, in drug development, the results obtained from animal studies can be pivotal to the further development of a therapy (39).

Initially, there was the demonstration that CD4+ T cells and the Th1/Th2 paradigm were an important characteristic of the allergic animals' response to antigen (4,7,10,18,21). More recently, the importance of IL-4/IL-13 was noted with signaling that activates transcription factor STAT 6 and appears to simulate many of the essential features associated with asthma (18,20). From these rather simplistic beginnings, there is now an expanding list of potentially new therapeutic targets. So while the ability of animal models to successfully predict the clinical efficacy of asthma therapeutics is hotly debated, there remains a clear role for animal models to unravel the complex process of the inflamed lung (39).

3. BETA RECEPTOR AND ASTHMA

3.1. Beta-agonists and animal models

Beta receptor agonists have been studied in animal systems, specifically, isolated airway smooth muscle (40) where adrenal cortex extracts were shown to relax contracted airway smooth muscle. In spite of the clear clinical effectiveness of beta-agonists as a class of asthma therapeutics, the FDA in 1992 mandated that manufacturers assess the risk of all racemic mixtures of therapeutic agents. This has triggered a series of studies into whether or not the enantiomers of beta-agonists have a differential or negative effect. These studies were also launched to determine if S-albuterol had negative or deleterious effects. Again, the complexity that is faced in designing such studies includes the consideration of the appropriate dosing and delivery of the drugs in question. For example, most studies administer beta-agonists by systemic delivery when the most common means of beta-agonist delivery in the clinical setting is inhalational as either a nebulized liquid, metered dose inhaler, or a dry powder formulation (13). Whereas systemic administration yields a measureable drug level, there is little way of knowing what the drug levels are following delivery in an inhaled formulation unless blood samples and the appropriate analysis are done. This limitation should be borne in mind when considering the results from many published studies.

Animal studies clearly show that beta-agonists or symptomatic neural mediators quickly reverse or prevent bronchoconstriction that is induced by antigen (41,42), serotonin (43,44) or by bronchospastic agents using *in vitro* smooth muscle preparations (45) supporting the use of beta-agonists as a frontline drug in asthma. Indeed, for all these reasons, beta-agonists are an important class of drugs that are the most widely prescribed class to treat asthma, especially for acute episodes (13).

3.2. Differential effects of enantiomers of albuterol

The differential effects of enantiomers of beta-agonists have been studied in regards to lung function *in vivo* using a variety of animal models. However, the data to date are confusing. Racemic albuterol contains an equal molar mix of R- and S-enantiomers; the R-enantiomer binds with nearly 100-fold greater affinity than that of the S-enantiomer. Accordingly, the S-enantiomer was thought to be inert or ineffective due to low binding affinity. More recently, *in vitro* studies suggest the S-enantiomer may have other effects not mediated by the beta-adrenergic receptor. Many of these effects are thought to be deleterious (26,45-48). Perhaps the most intriguing study suggests that the S-enantiomer might induce cellular proliferation and, in turn, contribute to airway remodeling (49). Additionally, S-albuterol stimulates the release of inflammatory cytokines in some cells (26, 46-48). S-albuterol also exhibits a longer serum half-life (45,50) that might be an important contributing factor to the reported pro-inflammatory effects.

In vivo, the effects of beta-agonist enantiomers are even more unclear. Using a mouse model, Henderson *et al* (51) reported that both R- and S-albuterol, when given systemically with osmotic pumps, reduced airway inflammation (R more than S) and mucous gland hyperplasia (R comparable to S). Of the measured inflammatory cytokines, only IL-4 was reduced and only when animals were treated with R-albuterol. Using a measure of AHR (Penh) that at best might measure irritant nerve activity (52) but not airway mechanics (53), Henderson and colleagues showed S-albuterol but not R-albuterol actively increased airways "hyperresponsiveness" due to allergen challenge. This disassociation between function and inflammation is confusing in as much as these

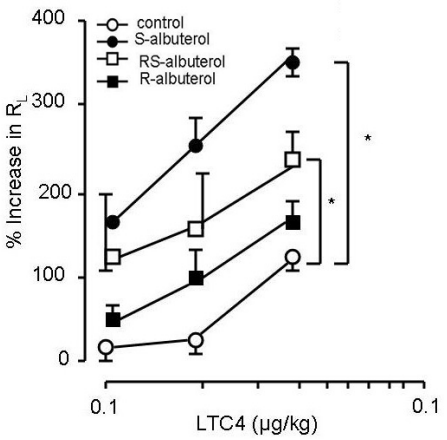

Figure 2. Differential effect of albuterol enantiomers on bronchoconstriction from leukotriene C_4 LTC_4 (adapted with permission from Keir and colleagues (54)). In naïve guinea pigs treated with RS-, R-, or S-albuterol for 10 days via an osmotic mini pump (1 mg·kg–1·d–1) or vehicle (0.9% saline). Bronchial hyperresponsiveness (BHR) to LTC_4 (measured as a percent increase in lung resistance) is greatest with S-albuterol and is not significantly changed by the R-enantiomer. *P < 0.05 versus saline (n = 6-8 in each treatment group) (54)

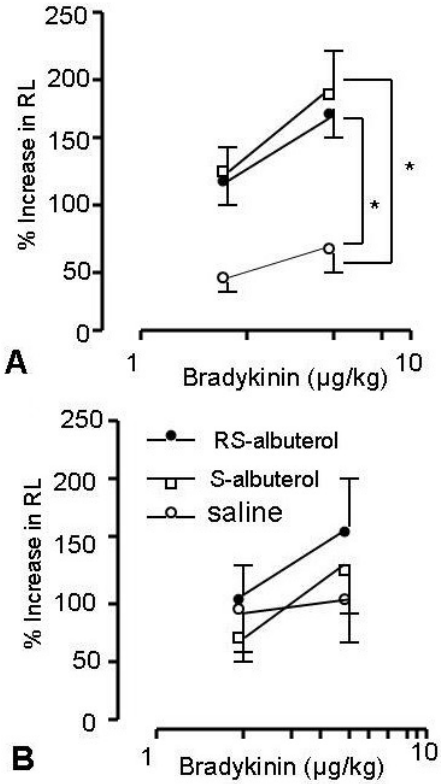

Figure 3. Capsaicin reduces the increase in bronchial hyperresponsiveness from albuterol in response to bradykinin. (adapted with permission from Keir and colleagues. (54)). In naïve guinea pigs treated with vehicle control (Panel A) or capsaicin (Panel B) for 3 days prior to a 10-day treatment with RS or S-albuterol. * P < 0.05 versus saline.

investigators had also shown that S-albuterol reduced airways inflammation that is generally considered the cause of AHR. Discerning the physiological impact of inflammatory changes combined with changes in mucus and airway liquid or edema in mouse asthma models and then extrapolating to humans with asthma has limitations but the data remain interesting.

The study of Kier and colleagues (54) implicating neural mechanisms may shed some further light on the controversy. In this study, guinea pigs were immunized and challenged with ovalbumin and treated systemically using mini osmotic pumps. In naïve animals, the racemic mix of albuterol increased airways response to histamines, bradykinin, but particularly notable was the increase in AHR to leukotriene LTC_4 (Figure 2). R-albuterol did not affect AHR and S-albuterol greatly enhanced AHR suggesting R-albuterol had a predominantly suppressive role. In the antigen-challenged animals, racemic albuterol increased the response to histamine and OVA *per se*. One might also conclude from these and other data that the mechanism of action of S-albuterol is distinct and unique from R-albuterol. As these adverse responses were all antagonized by chronic treatment with the neural toxic agent, capsaicin, a neural mechanism involving tachykinin receptors can be evoked (Figure 3). Capsaicin, when given chronically, depletes tachykinin-containing neurons. The guinea pig also has a very complex neural control system of its airways so it is appropriate to speculate that the complex actions of beta-agonists are in keeping with these species' complex neural control system. Earlier studies have used other beta-agonists such as isoprenaline and its enantiomers showing enhanced responses to histamine and bombesin (55,56); hence, these effects may be more of a class effect and not specific to albuterol alone.

Chronic beta-agonist treatment may also increase AHR by alternative mechanisms. In rats, it has been shown that AHR is enhanced possibly through a mechanism that involves goblet cell hyperplasia when beta-agonists are given to atopic animals (57-62). Similarly, we reported that chronic treatment with the long-acting beta-agonist, salmeterol, also increases AHR in antigen-challenged mice possibly via goblet cell hyperplasia (63). Since mucous glands and goblet cells respond to neural influences, there is the possibility that much of the deleterious effects of beta-agonists may be related to poorly understood effects of S-enantiomers on the neural influences on cells of the lung, inflammatory and/or to mucus-containing cells in particular. Clearly, more investigation is needed to define these deleterious effects; first because of the central role beta-agonists play in current asthma therapy, and second, because of the opportunity to develop new classes of beta-agonists free from these unwanted effects.

3.3. Beta receptor paradigm shift

Looking beyond the effects of the simple administration of beta-agonists or enantiomers, more detailed experiments examining the role of the beta-adrenoceptor in asthma suggests a potential paradigm shift. In cardiology, the thinking on therapeutic manipulation of the beta receptor has undergone recent dramatic changes

(64). In heart failure, chronic beta blockade is now widely practiced. In previous decades, this would have been unheard of given the concern that acute administration of beta blockers can cause cardiac decomposition (65). Similarly, in asthma treatment, there remains a firmly held belief that inhibiting the beta receptor is harmful because it causes bronchospasm (66). This thinking even extends into other airway diseases such as COPD. Paradoxically, chronic administration of beta blockers with agents such as nadolol (a non-selective beta-adrenoceptor (AR) antagonist or inverse agonist blocking both beta-1 and beta-2 receptors) reduces mucous gland hyperplasia and inflammation (67,68) . More recently, Nguyen *et al.* demonstrated reduced mucus metaplasia, AHR and inflammation when mice were treated chronically with the beta 2-AR inverse agonist and also in beta-2 AR deficient mice (69). The surprising conclusion of this study was that beta-2 AR receptor signaling is, in fact, required to produce the asthmatic phenotype. Furthermore, human clinical trials using the beta blocker nadolol in asthmatics have been reported with promising results (70). This is another example of the movement between human observations studied in the mouse model and then confirmed in careful study in human patients. This data suggests that although acutely, beta-agonists are an excellent bronchorelaxing agent, their effect on airway inflammation is becoming increasingly suspect. Understanding this potential problem may be an important focus of future asthma therapies and new beta-2 AR ligand therapeutics. It remains to be seen as to exactly where these current research directions will lead.

4. CONCLUSION

Animal models provide an important opportunity to investigate the mechanism of action by asthma therapeutics in the *in vivo* context. The current body of literature in this field is limited but suggests a complex mechanism of action of beta-agonists in the context of asthma. Indeed, it now seems unlikely that the efficacy of beta-agonists is due completely to relaxation of airway smooth muscle. The differential effect of the enantiomers of beta-agonists remains controversial, and S-enantiomers may account for many of the adverse effects observed with chronic beta-agonist usage. The data suggests further study into how beta-agonists affect neural control of the airways and mucus-containing cells is warranted. Clearly, animal models will remain an important and critical tool in the unraveling of both mechanisms of asthma pathogenesis and improving asthma treatment.

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Abbreviations: AHR: airways hyperresponsiveness, TH2: T-helper type 2;,AR: adrenoceptor

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