Current Issues With $\beta_2$-Adrenoceptor Agonists

Pharmacology and Molecular and Cellular Mechanisms

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$\beta_2$-Adrenoceptors are widely, almost ubiquitously, expressed. Activation of these receptors on bronchial smooth muscle by short- and long-acting $\beta_2$-adrenoceptor agonists causes bronchodilation. Here, the $\beta_2$-adrenoceptor is linked by the G protein, Gs, to adenylyl cyclase, which increases cyclic adenosine monophosphate (cAMP), thus activating protein kinase A, which affects calcium levels and reduces the efficiency of myosin light-chain kinase, causing relaxation. Activation also entrains numerous acute and longer term downregulation responses affecting the number, location, and net efficiency of signaling of the receptor. Synthetic $\beta_2$-agonists are all “partial agonists,” incompletely able to optimally stimulate cAMP signal transduction. However, compared with some cells (such as mast cells) involved in exercise-induced asthma induction, airway smooth muscle is privileged in that transduction efficiency is intrinsically high and the tissue is very resistant to complete downregulation. Glucocorticosteroids have broadly beneficial interactions with $\beta_2$-adrenoceptors. Researchers have recently discovered that the $\beta_2$-adrenoceptor may function as a homodimer and that it can form heterodimers with both the $\beta_1$- and $\beta_3$-adrenoceptors, and possibly other receptors. This further complicates interpretation of the effect of $\beta_2$-adrenoceptor polymorphisms, but it is unknown whether this occurs in humans in vivo. Researchers have known for some time that strong contraction involving receptors coupled to the Gq G protein (e.g., cholinergic and leukotriene receptors via negative biochemical crosstalk), virus infection (via uncoupling), and inflammation (via kinases) can impair relaxation. Most recently, researchers have discovered that the $\beta_2$-adrenoceptor can also send potentially adverse signals after “atypical coupling” to Gq rather than Gs. The clinical implications of these uncouplings, crosstalk, and atypical coupling possibilities are not well-understood.

Index Entries

$\beta_2$-Adrenoreceptor; lung; tachyphylaxis; signal transduction; LABAs.
**β₂-Adrenoceptor Distribution and Effects in Lung**

β₂-Adrenoceptor agonists (β₂-agonists) are clinically useful in the treatment of asthma, chronic obstructive pulmonary disease and numerous other respiratory diseases where they are used as bronchodilators. Traditionally, this ability to cause bronchodilation by relaxing airway smooth muscle has been seen as their single important mode of action. However, β₂-adrenoceptors are widely, almost ubiquitously, expressed on resident and structural cells of the lung and on the leukocytes that traffic through this organ (1,2). The effects of activation of these receptors have been extensively reviewed (3–9). Briefly, when expressed on airway smooth muscle, β₂-adrenoceptors mediate relaxation, but they can also suppress growth and may reduce inflammatory cytokine production in asthma. β₂-Adrenoceptors on post-capillary venules suppress plasma exudation in vivo and regulate fluid balance in alveolar epithelial cells. Sensory nerve activation, including antidromic reflexes, is dampened by β₂-agonists as is cholinergic neurotransmission, which is inhibited prejunctionally. Activation and, to a lesser extent, recruitment of most inflammatory leukocytes can be suppressed by β₂-agonists, at least acutely, but the evidence that this occurs in clinical asthma is weak or absent. Eosinophilic inflammation is scarcely affected, although suppression of neutrophilic inflammation has been observed (10,11).

Dendritic cells express β₂-adrenoceptors, and it is not widely appreciated that similarly to steroids, β₂-agonists promote T-helper 2 immune bias, which may contribute to early allergic asthma (7). Recent studies have suggested that by subtly increasing the residence time of activated glucocorticosteroid receptors in the cell nucleus, β₂-agonists may enhance their anti-inflammatory properties (12). This effect was first observed in fibroblasts but has now been demonstrated in human airway smooth muscle and epithelium, including epithelium infected with viruses implicated in acute exacerbations of asthma (13). Collectively, these effects would be broadly beneficial in asthma, although many of the nonairway smooth muscle effects are rapidly downregulated.

Remember also that β₂-adrenoceptors abound in the periphery (and brain). Inhaled β₂-agonists can affect the myocardium, where a subdominant population of β₂-adrenoceptors regulates rate and force. β₂-Adrenoceptors are also present in the atrial–ventricular conducting system and could conceivably impair pacemaker rhythm and conduction. β-Agonists can lengthen QTc intervals, but there is no clear evidence of association with Torsade de Pointes syndrome. In animals, β₂-agonists can be arrhythmogenic, but there has been no objective evidence of increased risk rate in humans without underlying cardiovascular disease or hypoxemia in the limited number of safety and Holter monitor studies that have been reported (14–17).

The safety and clinical efficacy record of β₂-agonists is enviable, particularly when combined with steroids (18). However, there remains a lingering suspicion that rarely, or perhaps in specific genetically susceptible subgroups, β₂-agonists may worsen control of disease and possibly increase the chance of severe asthma exacerbations and death when such exacerbations occur (19–21). This is notwithstanding that long-acting β₂-agonists (LABAs) plus steroid combinations have clearly, and quite dramatically, been proven to reduce the rate of exacerbations in numerous large and well-controlled clinical trials (22–24). This article summarizes some of the current evidence of how β₂-agonists work as bronchodilators in disease. It also highlights much less well-known aspects of the molecular and cellular pharmacology of β₂-adrenoceptors and their agonists that may inform the current debate.

**β₂-Agonists**

β₂-Agonists were originally developed by medicinal chemists based on the discovery of Alquist that catecholamines, such as adrenalin
(epinephrine), exerted their effects via distinct notional α- and β-receptors as well as the subsequent work by Lands, which split the notional β-adrenoceptors into β₁- and β₂-subdivisions. In 1987, the β₂-adrenoceptor was cloned, confirming this pharmacological classification and later confirming the existence of a third receptor that had been inferred from careful pharmacological studies, the β₃-adrenoceptor (which is essentially absent from the lung). The putative “β₄”-adrenoceptor is now believed to be an artifact representing the special state of the cardiac β₂-receptor.

By sequentially modifying the structure of catecholamines, medicinal chemists were able to improve β₂-adrenoceptor agonists selectivity, loosen susceptibility to degradation by catechol-O-methyl transferase and susceptibility to neuronal and extraneuronal uptake mechanisms, confer oral activity, and extend duration of action after inhalation. Short-acting β₂-agonists, such as salbutamol (albuterol) and terbutaline, belong to this lineage of agonists. Interestingly, the two most commonly used LABAs, salmeterol and formoterol (eformoterol), were developed almost contemporaneously with salbutamol and terbutaline along similar structure–activity principals. Subsequently, entirely novel chemical classes of β₂-agonists have been discovered by random screening, but these have not yet reached clinical practice. The older papers documenting the discovery and development of both formoterol and salmeterol are clear that both compounds were optimized for functional selectivity—that is, relaxation of airway smooth muscle vs chronotropic (rate) and ionotropic (force) effects in cardiac tissues, which is different from absolute receptor selectivity.

In the original papers, it is also clear that the long duration of action of both compounds was recognized early. Salmeterol was clearly optimized along a strategy to anchor the drug in or near the β₂-adrenoceptor (at a putative “exosite” or “exoceptor”) by extending its aliphatic side-chain—its active moiety is a saligenin head-group identical to salbutamol (25,26). Formoterol was optimized for potency, efficacy, and duration along separate lines of thought (27–29). Although it is most likely that the binding of salbutamol and terbutaline (and probably formoterol) rather closely—but not exactly—resembles the binding of adrenaline to β₂-adrenoceptor, the nature of salmeterol binding remains controversial. Based on functional observations, it had been proposed that long aliphatic side-chain of salmeterol bound to an accessory structure distinct from the β₂-adrenoceptor. Once the structure of the β₂-adrenoceptor was inferred from its homology to bacteriorhodopsin, whose absolute structure had been resolved by crystallography, it was clear that this concept was untenable.

However, the highly atypical binding and functional properties of salmeterol begged an explanation. Specifically, the property of salmeterol to resist being washed free of the receptor and its ability to “reassert” relaxation when its activity is transiently blocked by water-soluble β-antagonists remain fascinating. Two theories, which are not mutually exclusive, have been proposed to explain why LABAs are long-acting: (1) salmeterol’s tail binds to an internal accessory site deep in the core of the receptor (modified exosite model; ref. 30) and (2) the very high membrane partition affinity of salmeterol creates a microdepot of drug near the receptor (diffusion microkinetic model; ref. 31). The main difference between the drugs that is reasonable to accept is that salmeterol is intrinsically long-acting, whereas the duration of action of formoterol is critically dependent on its route of administration. Whereas salmeterol is long-acting both orally and by inhalation, formoterol shows a sustained duration of action only after inhalation. These discussions are not just academic; the exact nature of the sustained duration of individual LABAs is likely to relate to aspects of their clinical pharmacology and tolerability (32,33).
Pharmacologists describe agonists and antagonists with terms that are useful to understand the clinical properties of $\beta_2$-agonists: selectivity, specificity, potency, and efficacy (and intrinsic activity). Selectivity is now described in terms of ratios of absolute binding affinities to defined receptors in in vitro assays. However, all of the $\beta_2$-agonists in current clinical use were developed using classical assays of functional selectivity, where potency to relax airways smooth muscle (or in vivo bronchodilation) was compared with potency to increase the rate and/or force of cardiac tissue contraction in vitro (or the heart in vivo). The utility and predicative value of such assays is extended by the use of highly selective $\beta_1$- and $\beta_2$-adrenoceptor antagonists such as CGP 20712 ($\beta_1$) and ICI 118551 ($\beta_2$). The currently available short-acting $\beta_2$-agonists have very good functional (and receptor) selectivity for muscle relaxation vs heart effects, whereas the LABAs have very high selectivity (about 1000-fold) for $\beta_2$- vs $\beta_1$-adrenoceptor-mediated effects.

Potency is defined pharmacologically as the $-\log_{10}$ of the molar concentration of drug required to produce a half-maximal effect. Less readily understood is the concept of pharmacological efficacy, which is the degree of effect observed compared with the maximal possible effect in a system. Full agonists produce a full response and have an efficacy equal to 1 and partial agonists a result less than 1 but greater than 0. Neutral antagonists have an efficacy of 0. It is surprising to many that some drugs, called inverse agonists, have a negative efficacy ($<0$), because they are able to suppress spontaneous activation: in any pool of receptors there are always some in an active state, which occurs independently of an agonist being present. (This fact is very important for interpreting cell biology studies where the number of $\beta_2$-adrenoceptors is often craftily amplified well above physiological levels.)

Note that the efficacy of a drug depends on the systems in which it is tested—if receptors are highly abundant and well-coupled, partial agonists may appear to be full agonists. For example, salbutamol is a partial agonist on human airway smooth muscle in vitro but its peak in vivo bronchodilator response is not distinguishable from the full-agonist isoproterenol (isoproterenol) (34). Salmeterol is a weak partial agonist on human airway smooth muscle in vitro and behaves as an antagonist of $\beta_2$-agonists with higher efficacy (35), such as formoterol, but no such antagonism is demonstrable in vivo for bronchodilation. However, the partial-agonist effects of salmeterol on mast cells in human lung is demonstrable in humans in vivo (36).

$\beta_2$-Adrenoceptor Structure and Activation

The $\beta_2$-adrenoceptor is a member of the very large 7-transmembrane receptor superfamily of G protein-coupled receptors. It has proved impossible to resolve its fine structure by classical crystallography. However, it is highly homologous to other receptors, such as bacteriorhodopsin, whose structures have been solved. The $\beta_2$-receptor was the first receptor to be cloned (in 1987) and it has since been subjected to intense structure–activity mutagenesis studies. When these are combined with the vast body of pharmacological data on the binding properties of agonists (activators) and antagonists (blockers), sophisticated computer models have been proposed that probably most closely resemble its true structure (37). In these models, the receptor is composed of a single amino acid chain that forms 7 $\alpha$-helix coils localized to the membrane (transmembrane regions [TM]) connected by intra- and extracellular loops. A ligand binding pocket open to the extracellular space is formed when the 7 $\alpha$-helices of the receptor cluster together in a loose ring (38). The exterior N-terminus amino acid chain is modified by numerous sugar groups, which appear to be important in assisting the receptor in correctly inserting in the membrane and for receptor movements.
A palmitoylation site on the human receptor anchors it to the membrane, forming an additional short loop. Mutation of the palmitoylation site impairs some aspects of receptor function in vitro, such as coupling (40–42).

The ligand binding pocket for catecholamines involves an aspartate residue (amino acid Asp113, on TM3) and several critical serines (Ser 203, Ser 204, and Ser 207, all on TM5), which are believed to interact with the catechol group of adrenaline via hydrogen bonds (43). Together with interactions with Asn 293 (TM6), Ile 169 (TM4), Val 117 (TM3), and Phe 290 (TM6), these hydrogen bonds at Asp 113 and the TM5 serines are believed to cause subtle conformational changes that promote signaling (37,44). Interestingly, antagonists share some bonds, such as Asp 113, but do not occupy the same space as agonist. The binding of synthetic agonists (e.g., clinically useful bronchodilators) is believed to closely follow but not exactly mimic the catecholamine binding conformation of adrenaline (epinephrine). The fine structure of the receptor can also be altered by allosteric modifiers that bind away from the ligand pocket. Of these, the best characterized and most relevant to asthma (because its levels are altered by disease) is divalent zinc, which binds at His 269 bridging loops 5 and 6 (45,46).

Several single nucleotide polymorphisms are known to lead to subtle variations in the receptor structure and function. Their role in altering β2-adrenoceptor properties in asthma, and their role in asthma diathesis, remain controversial and is discussed at length in the article by Taylor in this issue. Interestingly, β2-adrenoceptor variants have been separately linked to obesity risk, which may be relevant. Of the known single nucleotide polymorphisms, only one, a polymorphism at Ile 164 (which is very rare clinically), directly affects binding of agonists: it specifically impairs salmeterol activity, and this is where the putative exosite may be located (47).

Although it has not been discussed in the respiratory literature, it has been suspected for about 10 yr and is now unequivocally confirmed (at least in cell lines and transgenic animal models) that the β2-adrenoceptor can form homodimers with itself (which may even be a preferred state) and heterodimers with other β-receptor subtypes. β2/2-Dimers may be physiologically required for optimal expression on the cell surface (48), and there is some evidence that this state is protected from downregulation (49). β1/2-Heterodimers were recently demonstrated in the hearts of transgenic mice in vivo (50). This may further and markedly complicate the analysis of the effects of β2-adrenoceptor polymorphisms and genetic haplotypes (patterns of inherited polymorphisms) because multiple species of receptor can be predicted to exist in a heterozygous patient. It is likely that the pharmacological behavior of a homodimer might not differ too markedly from a monomeric form, but the behavior of a β1/2-heterodimer is not likely to resemble either of the two component receptors. Heterodimers between the β1-adrenoceptor and β3-adrenoceptor have been demonstrated, whereas β2/3-heterodimers have only been observed in cell lines (50–52). The β1/2 heterodimer downregulates β2-agonist-induced internalization and coupling to the potentially pro-inflammatory ERK1/2 mitogen-activated protein family kinases (53). Theoretically, the β2-receptor could also couple with other G protein-coupled receptors, such as some serotonin or dopamine receptor subtypes. Additionally, oligomeric forms (i.e., >2 component β2-receptors in a functional cluster) are predicted as possible conformations (54).

**Signal Transduction**

Figure 1 shows an overview of transduction and regulation of the β2-adrenoceptor. Note that the β2-receptor is unlikely to have any fixed structure. Rather, it is believed to oscillate between different structural states. At rest, a small fraction of the β2-receptor population is always in an active signaling state, which accounts for basal cyclic adenos-
ine monophosphate (cAMP) production. Agonists act to increase the fraction of receptors in the active signaling conformation.

When the receptor is moved to an active state, its associated G protein trimer, called Gs, disassociates into a Gs subunit and a β/γ dimer. Gs binds to and activates adenyl cyclase, causing increased camp, which in turn activates protein kinase A (PKA) and probably also PKG. PKA phosphorylates protein substrates that control calcium availability and myosin light-chain kinase. Phosphorylated myosin light-chain kinase is ineffective in sustaining active tone (contraction) in airway smooth muscle, and therefore, the tissue relaxes passively. PKA is promiscuous and also phosphorylates the β2-receptor on serines in intracellular loop 3, contributing to desensitization.

Fig. 1. Schematic representation of β2-adrenoceptor (ADRB2) signaling and regulation. Bronchodilation. The ADRB2 is shown in an active state signaling via the G protein Gs to adenyl cyclase (AC) via cyclic adenosine monophosphate (cAMP; not shown) to activate protein kinase (PK)A and PKG. In turn, PKA (and perhaps PKG) phosphorylate substrates. Phosphorylation of myosin light-chain kinase MLCK reduces the efficiency of actin myosin interaction in the presence of reduced free calcium (both not shown) promoting bronchodilation. Downregulation. Phosphorylation of the ADRB2 together with the activity of G protein receptor kinase leads docking of β-arrestins, which allow other molecules to cluster to the ADRB2 by molecular scaffolding and promotes the internalization of the ADRB2 into endosomes (shown as a large circle). From the endosome, the ADRB2 may be destroyed internally, ubiquinylated, and shuffled to the 26 proteosome by E3 ligases for destruction (represented by punctate fragments) or recycled to the cell surface. Over the long term, further induction of phophodiesteases that degrade cAMP and reduction of ADRB2 mRNA contribute to further downregulation. Interactions with steroids. PKA can also target the glucocorticosteroids receptor, influencing gene transcription by increasing glucocorticosteroids receptor residency time in the nucleus. Crosstalk. Inflammatory and bronchoconstricting substances can activate phospholipase C and inositol trisphosphate via Gq. There is some evidence that the ADRB2 may inappropriately couple to Gq in disease (atypical coupling). This leads to functional antagonism (reduced potency and efficacy) of the Gs–AC–PKA pathway. The reciprocal can occur from ADRB2 to phospholipase C/inositol trisphosphate, but it is less strong than the effect of Gq-related pathways. Depression of the efficiency of ADRB2 signaling in this way by disease may be very important in moderate-to-severe asthma. Direct coupling. The ADRB2 can directly couple to some ion channels and proteins (represented by the two vertical ovals to the right of ADRB2). Arrows show stimulation or direction of movement. Dotted arrows show an abnormal process. Arrow ending in a bar denotes inhibition. + Denotes enhance, – denotes inhibit.
by reducing the coupling of Gs to adenylyl cyclase. The role of PKA and PKG in isoprenaline responses has been questioned (55), but the balance of current evidence indicates a critical role for PKA. Rho kinases, which are needed for contraction, are also targeted. Recent studies have demonstrated that the β2-adrenoceptor can also directly activate some transduction pathways, such as activation of the sodium–hydrogen exchanger regulatory protein without intermediary Gs protein (56,57). The receptor may also couple directly to potassium channels linked to relaxation of airway smooth muscle (58).

Shortly after activation by agonists, the β2-adrenoceptor is also phosphorylated by G protein receptor kinases (GRKs; GRK2 and GRK 5 are most important) on serines and threonines on its intracellular tail. This facilitates binding of β-arrestins. β-Arrestins have at least a dual role. First, they desensitize the receptor by uncoupling it from Gs, and they promote receptor internalization. Once internalized into these clathrin-coated endocytotic vesicles, the receptor may be recycled after enzymatic dephosphorylation or may be destroyed in lysosomes. Ubiquitinylated receptors can also be targeted for destruction to S26 proteosome by E3 ligases (59). Second, arrestsin have “scaffold” motifs in their structure; this serves as a bridge or binding intermediate for numerous secondary proteins that then cluster to the β2-adrenoceptor, including phosphodiesterase 4 (which hydrolyzes camp-weakening receptor signaling), some tyrosine kinase receptors (e.g., insulin and insulin-like growth factor), and other proteins (56,60).

Very recent research has shown that these phosphorylation events, which regulate the strength of receptor signaling, are also reversed without internalization and endosomal trafficking by phosphatases working at the inner cell membrane (61). This finding has questioned the long held view that resensitization of agonist-exposed receptors requires initial internalization and early endosome.

The β/γ dimmer dissociated form Gs is not silent—it may couple to inflammatory kinases, such as mitogen-activated protein kinase, and may co-activate AKT. There is also direct evidence that the β2-adrenoceptor can couple to other G proteins, therefore triggering entirely different transduction pathways. The most important known example is Gs coupling to PKC, a pathway usually associated with transduction from contractile (bronchoconstricting) and inflammatory mediator receptors.

Adaptations to Sustained
β2-Adrenoceptor Activation:
Tachyphylaxis, Tolerance,
and Compensation

Perhaps the most important current issue with the clinical use of β2-agonists is how prolonged use affects airway function. Studies of the effects of regular use of salbutamol as monotherapy have shown that compared with intermittent use, asthma control may deteriorate, but only in association with specific β2-adrenoceptor haplotypes. Repeat administration of all β2-agonists reduces peak bronchodilation in the initial phase of treatment, but after this initial step-down, the large residual response is stable for years. Similar slight step-downs in peak effect followed by very stable and sustained bronchodilation are also properties of LABAs. LABAs are usually recommended for maintenance treatment regimes. More tellingly, regular or sustained use of β-agonists causes a more marked step-down in bronchoprotection (i.e., suppression of induced bronchospasm) against inhaled methacholine or histamine that is observed in bronchodilation.

Note that bronchoprotection is not entirely lost; it resets at a lower, less efficacious level (22). Neither the step-down in bronchodilation nor bronchoprotection is progressive in long-term studies (62). β2-Agonists offer strong protection from mast cell degranulating indirect challenge agents such as adenosine, but this is markedly downregulated after regular use and
protection from exercise-induced asthma wanes (63). The propensity to lose protection has been linked to the Arg 16 polymorphism (64). Steroids are known to transcriptionally upregulate the \( \beta_2 \)-adrenoceptor number (there is a positive steroid response element in the \( \beta_2 \)-receptor gene promoter), and there is some evidence—but by no means consistency in results—that steroids can protect from loss of peak bronchodilation or the loss of bronchoprotection (65–67), particularly at higher doses and after oral dosing.

As described earlier, cells exposed to sustained \( \beta_2 \)-agonist stimulation show a typical pattern of response. Receptors are quite rapidly internalized from the cell surface, and surface expression eventually reaches a new equilibrium at reduced density (68). Biochemically, the receptor is phosphorylated by PKA and GRKs promoting internalization and uncoupling from Gs, recruitment of phosphodiesterase 4 (which hydrolyses cAMP), and an increased chance of coupling to Gq rather than Gs. Over a longer term, the levels of \( \beta_2 \)-adrenoceptor messenger RNA are reduced by induction of ribonucleases, and phosphodiesterase levels are upregulated (69,70).

Mutations that affect coupling to Gs affect downregulation but not sequestration (71), and interestingly, downregulation of signal does not necessarily depend on endocytosis (72). Downregulation of Gs is particularly important (73). The degree and consequences of these adaptations are highly cell-type-specific. Counterintuitively, there is no clear relationship between agonist potency or efficacy and propensity to downregulate \( \beta_2 \)-adrenoceptors: formoterol and salmeterol, which differ markedly in efficacy, behave in a near indistinguishable profile in this regard. The molecular basis for this is not known, but it is inferred that it may relate to additional functional states of the \( \beta_2 \)-adrenoceptor.

Researchers have long known that “cross-talk” from contractile agonist signaling, largely mediated via Gi and Gq G protein-coupled receptors (e.g., leukotriene cyst-LT1 and muscarinic M3 cholinceptors), functionally antagonizes \( \beta_2 \)-adrenoceptor responses in vitro and in vivo (74). As contraction increases, the concentration response curves for relaxation induced by all \( \beta_2 \)-agonists move to the right and collapse downward—that is, the drugs progressively lose potency and efficacy (69,70). This results from impairment of the \( \beta_2 \)-adrenoceptor and adenyl cyclase by IP3 and other intermediates of the Gi and Gq transduction pathways (74). Such impairment may be very important in uncontrolled asthma, where high levels of contractile agonists and acetylcholine from vago-vagal reflexes are likely to be present. It is also a contributing mechanism to the additive effect of anticholinergics (and antileukotrienes) to \( \beta_2 \)-agonist-induced bronchodilation. Additionally, inflammatory mediators, such as interleukin-1\( \beta \) and tumor necrosis factor-\( \alpha \), can uncouple the \( \beta_2 \)-adrenoceptor from its own transduction pathways (75). Such uncoupling has been observed in fatal human asthma where, paradoxically, \( \beta_2 \)-receptor numbers are increased (76,77).

The apparent ability of \( \beta_2 \)-agonists to increase the bronchoconstrictor potency of inhaled agonists is extremely interesting. Liggett (78), who has contributed some of the most provocative and original research to the field, has proposed that this may result from paradoxical signaling (78). This hypothesis is based on studies in mice, where the \( \beta_2 \)-adrenoceptor was transgenically overexpressed (which triggers sustained “signaling” because a small fraction of receptors is always in the “active” signaling state) or where the \( \beta_1 \) or \( \beta_2 \)-adrenoceptor was deleted by gene targeting (in rodents, \( \beta_1 \)-adrenoceptors can generate robust bronchodilator responses). Surprisingly, mice lacking \( \beta \)-receptors had reduced responses to the Gq-coupled constrictors methacholine, serotonin, and U46619 (which mimics thromboxane). IP3 (discussed earlier) as a mediator of functional antagonism...
was decreased in the knockouts and increased in the \( \beta_2 \)-adrenoceptor overexpressors, probably because of alterations in PLC \( \beta_1 \)-isoform (78). It is not known whether this result can be replicated with \( \beta \)-agonists, but interestingly, treatment of mice with \( \beta \)-antagonists showed decreased constrictor responses (79). These findings in mice are intriguing, but the mouse is a poor model of human airway smooth muscle behavior.

Collectively, this new information indicates that \( \beta_2 \)-adrenoceptor pharmacology is increasingly complex. When considering the additional complexity of genetic variations, not only in the receptor but also in its transduction intermediates, regulators, and modifiers, it is clear there will be great difficulty in resolving current issues in humans and that very large studies are needed. Nevertheless, this work needs to be performed to determine whether there is a means to identifying patient subpopulation(s) where the risk–benefit ratio of \( \beta_2 \)-agonists might be unacceptable.

References

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