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## Pepducins as a potential treatment strategy for asthma and COPD

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### Abstract

Current therapies to treat asthma and other airway diseases primarily include anti-inflammatory agents and bronchodilators. Anti-inflammatory agents target trafficking and resident immunocytes and structural cells, while bronchodilators act to prevent or reverse shortening of airway smooth muscle (ASM), the pivotal tissue regulating bronchomotor tone. Advances in our understanding of the biology of G protein-coupled receptors (GPCRs) and biased agonism offers unique opportunities to modulate GPCR function that include the use of pepducins and allosteric modulators. Recent evidence suggests that small molecule inhibitors of  $G\alpha_q$  as well as pepducins targeting  $G_q$ -coupled receptors can broadly inhibit contractile agonist-induced ASM function. Given these advances, new therapeutic approaches can be leveraged to diminish the global rise in morbidity and mortality associated with asthma and chronic obstructive pulmonary disease.

### Summary

Despite considerable research efforts, asthma and COPD remain substantial therapeutic challenges and contribute globally to increasing morbidity and mortality. Bronchodilators reverse or prevent shortening of airway smooth muscle, and remain principal drugs in both prophylactic management and treatment of obstructive airway diseases. Over the past two decades, advances in management of the non-severe asthma/COPD patient have been limited to modest refinements in traditional bronchodilator drugs. Recent advances in GPCR biology and structural protein biochemistry offer

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unparalleled opportunities to develop novel therapeutic approaches that either activate or inhibit GPCR function. Coupling these discoveries to leveraging delivery systems that specifically target the airways may profoundly affect the morbidity and mortality in airway diseases.

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## Introduction

Profound advances in the fields of receptor biology and pharmacology have now identified novel approaches to modulate the function of G protein-coupled receptors (GPCRs), the largest super family of cell-surface receptors in the human genome [1,2]. Although the concept of rational drug design has existed for decades, only recently have structural biology and molecular modeling capabilities advanced this concept beyond the theoretical stage. Providing a further boost is the related and exploding field of biased ligand pharmacology, whose insights have greatly advanced our understanding of qualitative GPCR signaling. One intriguing discovery 16 years ago was the identification that receptor intracellular surface loops that interact with G proteins can be targeted by N-terminal lipidated peptides (pepducins) to modulate GPCR signaling and potentially serve as therapeutics [1,3].

Current therapies to treat asthma and other airway diseases primarily include anti-inflammatory agents and bronchodilators. Anti-inflammatory agents target trafficking and resident immunocytes and structural cells, while bronchodilators act to prevent or reverse shortening of airway smooth muscle (ASM), the pivotal tissue regulating bronchomotor tone. Unfortunately, about 50% of patients with asthma have inadequate control with current therapeutics [4]. Despite some refinements in the duration and specificity of  $\beta_2$ -adrenergic receptor ( $\beta_2$ AR) agonists and in the use of antagonists of contractile GPCRs in asthma, continued refinement may yield diminishing returns and truly novel approaches will be needed to overcome current limitations and address the needs of patients not served by currently available therapies.

Most bronchodilators are GPCR ligands which exert their action by either promoting pro-relaxant or inhibiting pro-contractile signaling. This underscores the importance of the competitive balance of pro-relaxant and pro-contractile GPCR signaling in regulating ASM contractility and airway resistance in obstructive lung diseases [5–7]. Airway resistance is also affected by structural changes in the airways (airway remodeling) and pulmonary architecture as well as tissue mechanics, but GPCR ligands acting on ASM have the strongest impact on changes in airflow.

Increased levels of endogenous contractile GPCR agonists have been associated with allergic airway inflammation, leading to increased ASM contraction and airflow obstruction. One strategy to ameliorate this is to inhibit pro-contractile GPCR signaling by administering a small molecule antagonist of a pro-contractile receptor. This approach may be suboptimal or entirely ineffective when multiple GPCRs are activated to contract ASM. Treatment with  $\beta$ -agonists relaxes airways by antagonizing pro-contractile GPCRs at several loci in airway smooth muscle including transmembrane receptor signaling, calcium mobilization and flux, and distal regulation of contractile filaments through regulation of myosin light chain kinase/phosphatase (MLCK/MLCP) activity. Therefore, use of  $\beta$ -agonists has an inherent advantage over selective antagonists of pro-contractile GPCRs as  $\beta$ -agonists dilate/protect

airways irrespective of the type of pro-contractile stimulus. Even though inhaled  $\beta$ -agonists are the mainstay therapy for acute bronchospasm and combined long-acting  $\beta$ -agonist (LABA) and corticosteroid treatment is the cornerstone of asthma and chronic obstructive pulmonary disease (COPD) maintenance therapy, concerns about  $\beta$ -agonist efficacy and safety have persisted for decades. Chronic  $\beta$ -agonist use has been associated with  $\beta_2$ AR tachyphylaxis [8–10], loss of asthma control [11–13], and death [14–17]. Although associations of chronic  $\beta$ -agonist use with adverse events have not been uniquely observed in all studies [18–20], the need for understanding mechanisms involved in detrimental effects of  $\beta$ -agonists on lung physiology and pathology remains.

Fortunately, recent basic science research in GPCR biology and pharmacology has discovered novel modes of GPCR signaling and regulation that potentially explains the complex effects of existing drugs while offering the promise of new, more efficacious drugs. In this review, we summarize studies based on newfound paradigms in GPCR biology to (1) identify strategies capable of making existing asthma and COPD drugs better; and (2) develop new drugs that work by distinctly different mechanisms.

### **Diverse receptor conformations are linked to differential signaling events; arrestins and biased ligands can influence the qualitative nature of signaling**

GPCR ligands are often characterized based on their efficacy, be it positive or negative, to a specific signaling pathway that is in turn linked to a specific cellular function. However, it has been apparent for some time that there exists a much greater complexity of ligand-receptor interactions, and recent studies that attempt to explain ‘why’ are probably only scratching the surface of the regulatory phenomena that enable receptor dexterity. Numerous GPCRs exhibit significant diversity in their capacity to activate multiple signaling pathways, linked to either other G proteins or in a G protein-independent manner. Some ligands engage a receptor to induce a distinct receptor conformation capable of activating one signaling pathway while inhibiting another pathway. This phenomenon, referred to ‘functional selectivity’ or ‘biased agonism’, has been demonstrated for a number of GPCRs. The 5-HT<sub>2C</sub>R ligand SB 242084, promoted 5-HT<sub>2C</sub>R-mediated PLC activation [21] but inhibited 5-HT<sub>2C</sub>R-mediated activation of phospholipase A<sub>2</sub> (PLA<sub>2</sub>) and the subsequent release of arachidonic acid (AA)[22]. In a somewhat striking observation, studies by Stout *et al.* [23] show that the agonist efficacy of ligands does not correlate with the capacity to induce desensitization of the different signaling pathways, therefore suggesting differential regulation of desensitization by various ligands of the 5-HT<sub>2C</sub>R. Similar observations have been made for 5-H<sub>2A</sub>,  $\mu$ -opioid, somatostatin, and chole-cystokinin receptors, where the level of efficacy of various ligands is dissociated from the ability to promote agonist-induced receptor desensitization (reviewed in [24]). Studies of D<sub>2</sub> [25] and D<sub>1</sub> dopamine [26], H<sub>1</sub> histamine [27] and V<sub>2</sub> vasopressin [24,28] receptors have identified ligands that exhibit differential abilities in pathway activation and receptor desensitization and trafficking.

Agonism bias is also exhibited towards G protein-independent signaling. Arrestins have now become established as the most prominent G protein-independent signaling molecules

(Figure 1). Arrestins were initially characterized as important regulators of receptor desensitization and internalization; arrestins are recruited to activated GPCRs where they subsequently hinder G protein recruitment and facilitate receptor internalization via clathrin-coated pits [29]. More recently arrestins have been proposed to act as signaling scaffolds and a large body of evidence now supports the capacity of various ligands to promote  $\beta$ -arrestin-dependent signaling events in absence of G protein activation. ICI 118,551 and propranolol, both inverse agonists for  $\beta_2$ AR-mediated  $G_s$  activation, have been shown to induce  $\beta_2$ AR-mediated arrestin-dependent p42/p44 activation [30]. This differential efficacy has been observed for other receptors including the V2 vasopressin receptor [30] and the angiotensin-1 receptor [31]; which both have the ability to activate p42/p44 via  $\beta$ -arrestin recruitment independent of  $G_q$  activation when bound to their respective biased ligands.

Collectively, these findings demonstrate that GPCR ligands can induce a range of distinct receptor conformations which determine signaling outcomes. Wisler *et al.* [32] identified carvedilol as having a unique profile out of a panel of 16  $\beta$ AR ligands: it is an inverse agonist for  $\beta$ AR-mediated  $G_s$  signaling but stimulates receptor phosphorylation, recruits beta-arrestin, promotes receptor internalization and activates p42/p44 in an arrestin-dependent manner. The authors hypothesize that this unique profile contributes to the effectiveness of carvedilol in treatment of heart failure. Current evidence suggests that biased agonism, through either choice of orthosteric ligands, use of allosteric modulators, direct targeting of G proteins, or manipulation of GRK and arrestin function, can be realized to tailor GPCR function and improve our ability to modulate airway inflammation and/or promote bronchodilation.

## **Novel strategies co-opting heterotrimeric G protein regulatory mechanisms can regulate ASM contraction**

GPCRs in ASM represent attractive therapeutic targets for multiple reasons, including the ability to readily target them with selective agents (inhaled small molecules). Approaches for targeting downstream elements have some conceptual appeal; for example, broad-based strategies for inhibiting the heterotrimeric  $G_q$  protein could be an effective means of negating the effects of all pro-contractile agonists in the inflamed airway, and overcoming the limitations of monotherapies such as tiotropium and montelukast [33]. To date, however, such approaches have not materialized as sufficiently selective and deliverable reagents have not been developed. However, recent studies have determined experimental approaches for manipulating heterotrimeric G protein function, many discovered as a consequence of our improved understanding of GPCR-G protein coupling, RGS function, and structural biology of heterotrimeric G proteins. We feel such approaches can be adapted to inhibit  $G_q$  signaling, and consequently  $G_q$ -mediated contraction, in ASM.

Multiple strategies designed to interdict GPCR- $G_q$  or  $G_q$ -PLC coupling as a means of global inhibition of  $G_q$ -coupled receptor signaling may offer new therapeutic approaches in airway diseases. Strategies include pepducins (Figure 2) that function as broad-based antagonists of  $G_q$  signaling, lipidated  $G\alpha_q$  peptides that selectively disrupt GPCR/ $G_q$  coupling, and small molecule inhibitors of  $G_{\beta\gamma}$ -mediated signaling. Importantly, most of these strategies employ

lipidated peptides that are amenable to delivery to the airway and ASM cells, and are more likely to specifically engage their target than are gene delivery approaches that are often required for targeting protein–protein interactions.

Our initial efforts to bias signaling with lipidated peptides focused on the development of pepducins derived from the intracellular regions of a GPCR [1]. The Benovic laboratory found that pepducins from the third intracellular loop (ICL3) of the  $\beta_2$ AR could mediate  $G_s$ -biased signaling [34] while pepducins from ICL1 mediated arrestin-biased signaling [35]. Some ICL3 pepducins were found to stabilize a conformation of the  $\beta_2$ AR that interacted selectively with  $G_s$  while other ICL3 pepducins directly activated  $G_s$ . While receptor-specific ICL3 pepducins had properties attributable to  $G_s$ -biased signaling such as reduced  $\beta_2$ AR endocytosis and desensitization, these molecules were not effective at attenuating carbachol-induced contraction in human precision-cut lung slices [36]. Nevertheless, given the ability of pepducins to bias  $G_s$ -signaling, efforts are underway to identify small molecules that would have a similar bias. Initial efforts should involve screening compounds using the  $\beta_2$ AR as a target to identify  $G_s$ -biased agonists,  $G_s$ -biased positive allosteric modulators and arrestin-biased negative allosteric modulators.

While stimulation of  $G_s$  signaling serves as a primary strategy for treating asthma,  $G_q$  signaling is also an important target. However, while inhibiting human ASM contraction can serve as an effective means of treating asthma, one problem is that there are many  $G_q$ -coupled receptors that are activated in asthma or COPD including the  $M_3$  muscarinic acetylcholine, cysteinyl leukotriene, H1 histamine, thromboxane, endothelin-A/B and others. To assess whether broad inhibition of  $G_q$  signaling is effective at attenuating ASM contraction, Carr *et al.* utilized several strategies to inhibit  $G_q$  including the  $G_{\alpha_q}$  specific inhibitor FR900359 (aka UBO-QIC) and a  $G_q$ -coupled receptor pepducin that appears to function as an antagonist [36]. FR900359 was confirmed as a selective  $G_q$  inhibitor which does not substantially effect  $G_s$ ,  $G_i$  or  $G_{12/13}$  activity. Both the P4pal-10 pepducin and the small molecule FR900359 were effective at inhibiting  $G_q$ -mediated signaling and growth in primary HASM cells, while only FR900359 effectively inhibited agonist-promoted airway contraction in human precision cut lung slices [36]. In addition the pepducin P4pal-10, derived from the intracellular loop of the protease-activated receptor (PAR) 4, was determined to function as a broad-based  $G_q$  inhibitor in HEK293 and human ASM cells (Figure 3; data from Carr *et al.* [36].) P4pal-10 was shown to inhibit  $G_q$  coupling to receptors including PAR1,  $M_3$  muscarinic acetylcholine ( $M_3$  mAChR), and H1 histamine (H1R), whereas no direct effects on  $G_q$  or the ability to antagonize GPCRs coupled to  $G_s$  were observed. Consistent with its ability to inhibit  $M_3$  mAChR and histamine H1R coupling to  $G_q$ , p4Pal-10 inhibited ASM contraction induced by carbachol and histamine. These studies, using two approaches to inhibit  $G_q$  signaling, indicate that inhibition of  $G_q$  signaling at the receptor locus may be a feasible approach to treat several pathophysiological processes in airway disease.

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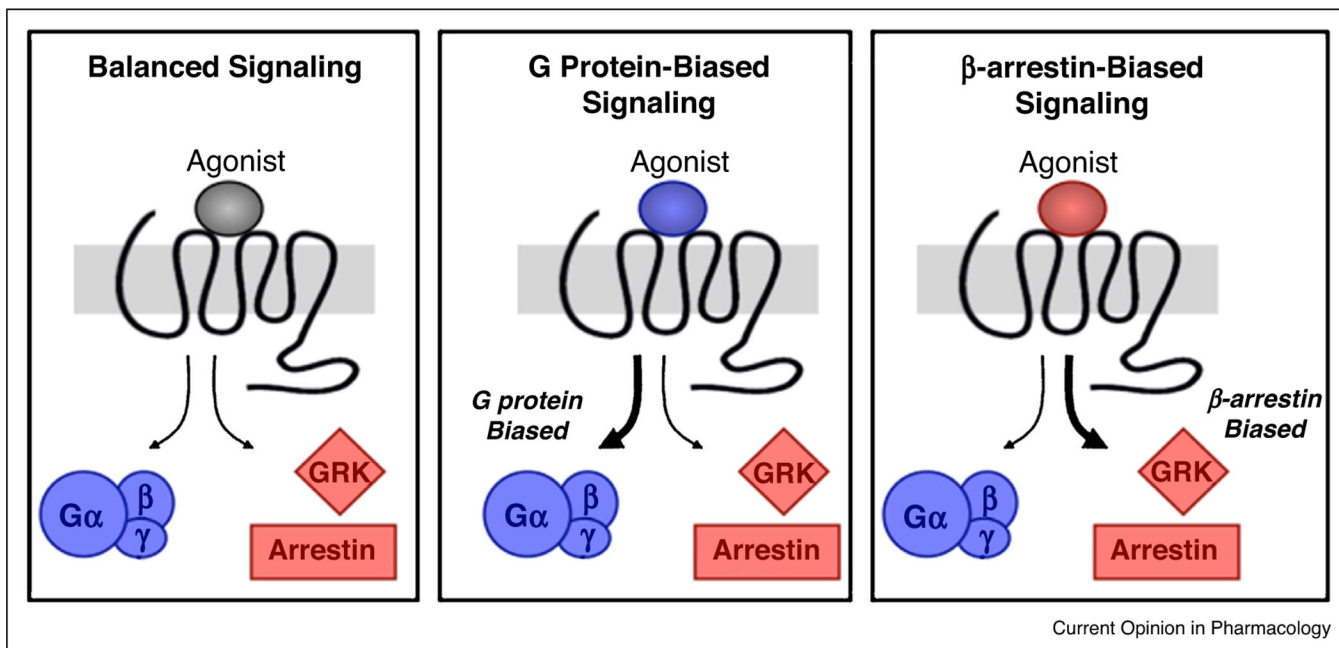


## References

1. Carr R, 3rd, Benovic JL: From biased signalling to polypharmacology: unlocking unique intracellular signalling using pepducins. *Biochem Soc Trans* 2016, 2:555–561.
2. Fredriksson R, Lagerstrom MC, Lundin LG, Schiöth HB: The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol Pharmacol* 2003, 6:1256–1272.
3. Covic L, Gresser AL, Talavera J, Swift S, Kuliopulos A: Activation and inhibition of G protein-coupled receptors by cell-penetrating membrane-tethered peptides. *Proc Natl Acad Sci U S A* 2002, 2:643–648 117359.
4. Peters SP, Jones CA, Haselkorn T, Mink DR, Valacer DJ, Weiss ST: Real-world Evaluation of Asthma Control and Treatment (REACT): findings from a national Web-based survey. *J Allergy Clin Immunol* 2007, 6:1454–1461.
5. McGraw DW, Liggett SB: Molecular mechanisms of beta2-adrenergic receptor function and regulation. *Proc Am Thorac Soc* 2005, 4:2713324 292–296; discussion 311–292.
6. Penn RB, Benovic JL: Regulation of heterotrimeric G protein signaling in airway smooth muscle. *Proc Am Thorac Soc* 2008, 1:47–57 2645302.
7. Penn RB, Pascual RM, Kim YM, Mundell SJ, Krymskaya VP, Panettieri RA, Jr, Benovic JL: Arrestin specificity for G protein-coupled receptors in human airway smooth muscle. *J Biol Chem* 2001, 35:32648–32656.
8. Newnham DM, Grove A, McDevitt DG, Lipworth BJ: Subsensitivity of bronchodilator and systemic beta 2 adrenoceptor responses after regular twice daily treatment with eformoterol dry powder in asthmatic patients. *Thorax* 1995, 5:497–504 1021218.
9. O'Connor BJ, Aikman SL, Barnes PJ: Tolerance to the nonbronchodilator effects of inhaled beta 2-agonists in asthma. *N Engl J Med* 1992, 17:1204–1208.
10. Vathenen AS, Knox AJ, Higgins BG, Britton JR, Tattersfield AE: Rebound increase in bronchial responsiveness after treatment with inhaled terbutaline. *Lancet* 1988, 8585:554–558.
11. Salpeter SR, Buckley NS, Ormiston TM, Salpeter EE: Meta-analysis: effect of long-acting beta-agonists on severe asthma exacerbations and asthma-related deaths. *Ann Intern Med* 2006, 12:904–912.
12. Sears MR: Adverse effects of beta-agonists. *J Allergy Clin Immunol* 2002, 6(Suppl.):S322–S328.
13. Sears MR, Taylor DR, Print CG, Lake DC, Li QQ, Flannery EM, Yates DM, Lucas MK, Herbison GP: Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet* 1990, 8728:1391–1396.
14. Castle W, Fuller R, Hall J, Palmer J: Serevent nationwide surveillance study: comparison of salmeterol with salbutamol in asthmatic patients who require regular bronchodilator treatment. *BMJ* 1993, 6884:1034–1037 1676982.
15. Nelson HS, Weiss ST, Bleecker ER, Yancey SW, Dorinsky PM, Group SS: The Salmeterol Multicenter Asthma Research Trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol. *Chest* 2006, 1:15–26.
16. Pearce N, Beasley R, Crane J, Burgess C, Jackson R: End of the New Zealand asthma mortality epidemic. *Lancet* 1995, 8941:41–44.
17. Spitzer WO, Suissa S, Ernst P, Horwitz RI, Habbick B, Cockcroft D, Boivin JF, McNutt M, Buist AS, Rebeck AS: The use of beta-agonists and the risk of death and near death from asthma. *N Engl J Med* 1992, 8:501–506.
18. Bateman E, Nelson H, Bousquet J, Kral K, Sutton L, Ortega H, Yancey S: Meta-analysis: effects of adding salmeterol to inhaled corticosteroids on serious asthma-related events. *Ann Intern Med* 2008, 1:33–42.
19. Dennis SM, Sharp SJ, Vickers MR, Frost CD, Crompton GK, Barnes PJ, Lee TH: Regular inhaled salbutamol and asthma control: the TRUST randomised trial. Therapy Working Group of the National Asthma Task Force and the MRC General Practice Research Framework. *Lancet* 2000, 9216:1675–1679.
20. Drazen JM, Israel E, Boushey HA, Chinchilli VM, Fahy JV, Fish JE, Lazarus SC, Lemanske RF, Martin RJ, Peters SP, Sorkness C, Szeffler SJ: Comparison of regularly scheduled with as-needed

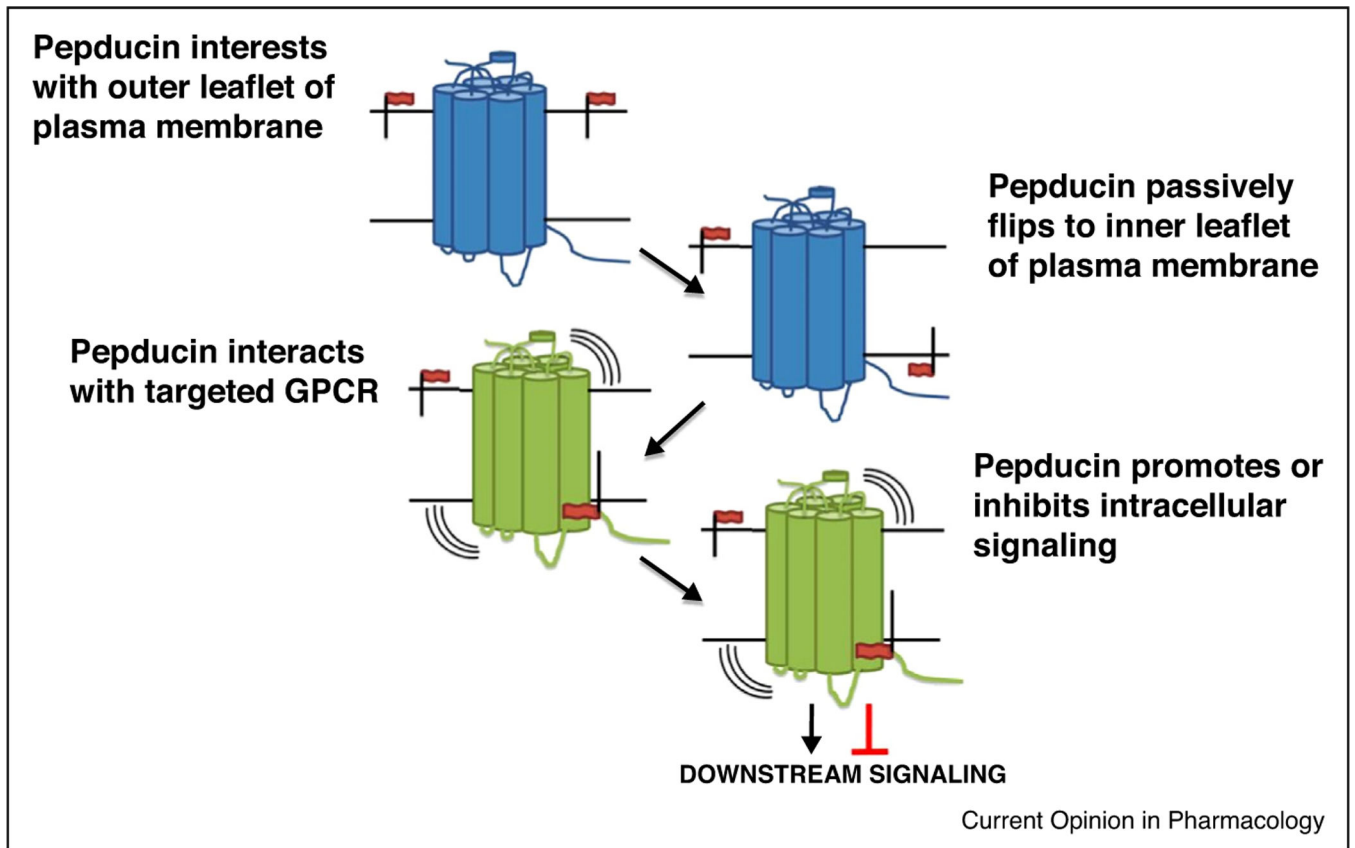
- use of albuterol in mild asthma. Asthma Clinical Research Network. *N Engl J Med* 1996, 12:841–847.
21. Cussac D, Newman-Tancredi A, Duqueyroi D, Pasteau V, Millan MJ: Differential activation of Gq/11 and Gi(3) proteins at 5-hydroxytryptamine(2C) receptors revealed by antibody capture assays: influence of receptor reserve and relationship to agonist-directed trafficking. *Mol Pharmacol* 2002, 3:578–589.
  22. De Deurwaerdere P, Navailles S, Berg KA, Clarke WP, Spampinato U: Constitutive activity of the serotonin<sub>2C</sub> receptor inhibits in vivo dopamine release in the rat striatum and nucleus accumbens. *J Neurosci* 2004, 13:3235–3241.
  23. Stout BD, Clarke WP, Berg KA: Rapid desensitization of the serotonin(2C) receptor system: effector pathway and agonist dependence. *J Pharmacol Exp Ther* 2002, 3:957–962.
  24. Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H, Javitch JA, Roth BL, Christopoulos A, Sexton PM, Miller KJ, Spedding M, Mailman RB: Functional selectivity and classical concepts of quantitative pharmacology. *J Pharmacol Exp Ther* 2007, 1:1–13.
  25. Gay EA, Urban JD, Nichols DE, Oxford GS, Mailman RB: Functional selectivity of D2 receptor ligands in a Chinese hamster ovary hD2L cell line: evidence for induction of ligand-specific receptor states. *Mol Pharmacol* 2004, 1:97–105.
  26. Ryman-Rasmussen JP, Nichols DE, Mailman RB: Differential activation of adenylate cyclase and receptor internalization by novel dopamine D1 receptor agonists. *Mol Pharmacol* 2005, 4:1039–1048.
  27. Moniri NH, Covington-Strachan D, Booth RG: Ligand-directed functional heterogeneity of histamine H1 receptors: novel dual-function ligands selectively activate and block H1-mediated phospholipase C and adenylyl cyclase signaling. *J Pharmacol Exp Ther* 2004, 1:274–281.
  28. Barak LS, Oakley RH, Laporte SA, Caron MG: Constitutive arrestin-mediated desensitization of a human vasopressin receptor mutant associated with nephrogenic diabetes insipidus. *Proc Natl Acad Sci U S A* 2001, 1:93–98 14550.
  29. Shenoy SK, Lefkowitz RJ: Seven-transmembrane receptor signaling through beta-arrestin. *Sci STKE* 2005, 308:cm10.
  30. Azzi M, Charest PG, Angers S, Rousseau G, Kohout T, Bouvier M, Pineyro G: Beta-arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G protein-coupled receptors. *Proc Natl Acad Sci U S A* 2003, 20:11406–11411 208770.
  31. Wei H, Ahn S, Shenoy SK, Karnik SS, Hunyady L, Luttrell LM, Lefkowitz RJ: Independent beta-arrestin 2 and G protein-mediated pathways for angiotensin II activation of extracellular signal-regulated kinases 1 and 2. *Proc Natl Acad Sci U S A* 2003, 19:10782–10787 196880.
  32. Wisler JW, DeWire SM, Whalen EJ, Violin JD, Drake MT, Ahn S, Shenoy SK, Lefkowitz RJ: A unique mechanism of beta-blocker action: carvedilol stimulates beta-arrestin signaling. *Proc Natl Acad Sci U S A* 2007, 42:16657–16662 2034221.
  33. Penn RB: Embracing emerging paradigms of G protein-coupled receptor agonism and signaling to address airway smooth muscle pathobiology in asthma. *Naunyn Schmiedebergs Arch Pharmacol* 2008, 2:149–169.
  34. Carr R, 3rd, Du Y, Quoyer J, Panettieri RA, Jr, Janz JM, Bouvier M, Kobilka BK, Benovic JL: Development and characterization of pepducins as Gs-biased allosteric agonists. *J Biol Chem* 2014, 52:35668–35684 4276837.
  35. Carr R, 3rd, Schilling J, Song J, Carter RL, Du Y, Yoo SM, Traynham CJ, Koch WJ, Cheung JY, Tilley DG, Benovic JL: Beta-arrestin-biased signaling through the beta<sub>2</sub>-adrenergic receptor promotes cardiomyocyte contraction. *Proc Natl Acad Sci U S A* 2016, 28:E4107–E4116 PMC4948363.
  36. Carr R, 3rd, Koziol-White C, Zhang J, Lam H, An SS, Tall GG, Panettieri RA, Jr, Benovic JL: Interdicting Gq activation in airway disease by receptor-dependent and receptor-independent mechanisms. *Mol Pharmacol* 2016, 1:94–104 4702101.





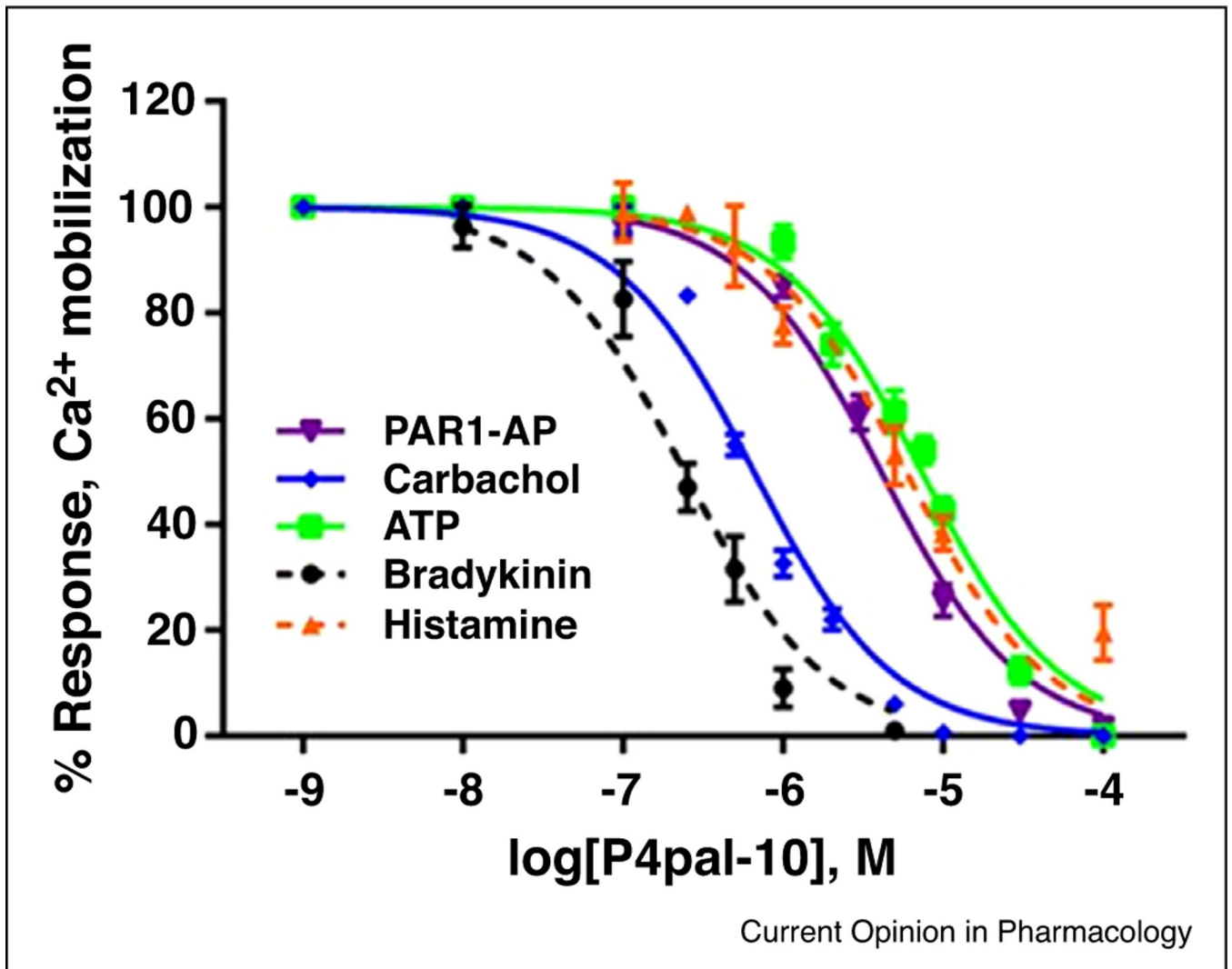
**Figure 1.**

General concept of biased agonism. An unbiased agonist will induce balanced signaling by promoting coupling to G proteins and GRKs/  $\beta$ -arrestins. This typically will promote G protein-dependent and  $\beta$ -arrestin-dependent signaling as well as GPCR desensitization, endocytosis and degradation. In contrast, a biased agonist will induce a receptor conformation that will enable selective activation of G protein — dependent or G protein-independent (arrestin) signaling. In addition, for those GPCRs capable of coupling to multiple G proteins, biased ligands may preferentially promote GPCR coupling to a specific G protein. Accordingly, biased agonism can dramatically influence the functional consequences of receptor activation, and influence receptor desensitization.



**Figure 2.**

A proposed mechanism of action for pepducin effects on GPCR function. The lipidated nature of the peptides fosters pepducin incorporation into the outer leaflet of the plasmalemma. The fluidity of the pepducin to move within the plasma membrane can stabilize the GPCR conformation and/ or prevent G protein interaction to promote or inhibit intracellular signaling [1].



**Figure 3.** Pepducins as inhibitors of GPCR-Gq signaling. Agonist-promoted calcium mobilization was monitored by Fura-2 AM fluorescence in agonist-stimulated HEK293 (PAR1-AP, ATP, Carbachol) and human ASM cells (Bradykinin, Histamine) pretreated for 1 min with indicated concentrations of P4pal-10. Data from Carr *et al. Mol Pharmacol* 2016; values are mean  $\pm$  SD,  $n = 3$ .