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Functional Selectivity of GPCR Ligand Stereoisomers: New Pharmacological Opportunities

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Running title

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Abbreviations

β_xAR, β_x-adrenergic receptor; GPCR, G-protein-coupled receptor; G-protein, heterotrimeric GTP-binding protein consisting of an α-subunit and a $\beta\gamma$-complex; G_i, inhibitory G-protein of adenylyl cyclase; G_s, stimulatory G-protein of adenylyl cyclase; G_q, stimulatory G-protein of phospholipase C.

The terms activity / potency and active / potent are used as synonyms.

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Abstract

It is now well established that any given ligand for a G-protein coupled receptor (GPCR) does not simply possess a single defined efficacy. Rather, a ligand possesses multiple efficacies, depending on the specific down-stream signal transduction pathway analyzed. This diversity may be based on ligand-specific GPCR conformations and is often referred to as “Functional Selectivity”. It has been known for a century that stereoisomers of catecholamines differ in their potency, and in some systems, also in their efficacy. However, the molecular basis for efficacy differences of GPCR ligand stereoisomers has remained poorly defined. In an elegant study published in this issue of *Molecular Pharmacology*, Woo et al. show that stereosiomers of the β_2-adrenoceptor selective agonist fenoterol differentially activate G_s- and G_i-proteins in native rat cardiomyocytes. This study is so important because it is the first report to show that even the subtle structural differences within a ligand stereoisomer pair are sufficient to discriminate between GPCR conformations with distinct G-protein coupling properties. The study highlights of how important it is to examine the “more active” (eutomer) and the “less active” (distomer) stereoisomer to understand the mechanisms of action and the cellular effects of GPCR ligands. The study by the Xiao group will ignite a renaissance of the analysis of ligand stereoisomers, using sensitive pharmacological and biophysical assays. The available literature supports the notion that meticulous analysis of ligand stereoisomers is a goldmine for understanding mechanisms of GPCR activation, analysis of signal transduction pathways, development of new therapies for important diseases and drug safety.

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The concept of functional selectivity. The two-state model assumes that GPCRs exist in an inactive (R) state and an active R* state. Binding of an agonist to GPCR stabilizes the active R* conformation in the receptor, allowing it to promote GDP/GTP exchange at G-proteins and subsequent activation (or inhibition) of cellular effector systems (Gether and Kobilka, 1998; Seifert and Wenzel-Seifert, 2002). Partial agonists exhibit lower efficacy than full agonists at stabilizing the R* state and, accordingly, are less efficacious at promoting G-protein activation. However, during the past decade, it has become increasingly clear from pharmacological and biophysical studies that agonists actually possess multiple efficacies, depending on the specific G-protein to which the GPCR is coupled and the particular down-stream signal transduction pathway analyzed. Thus, instead of a single active R* state, multiple ligand-specific active GPCR conformations (R^{*1}-R^{*n}) may exist (Fig. 1). Classic pharmacological concepts did not predict this diversity and complexity in agonist action which is currently often referred to as “Functional Selectivity”. This issue has been the subject of a number of excellent recent reviews by leading groups (Perez and Karni, 2005; Milligan et al., 2007; Urban et al., 2007; Neubig, 2007; Kenakin, 2007; Kobilka and Deupi, 2007; Galandrin et al., 2007; Lohse et al., 2008).

The β_2AR, a dually G_s- and G_i-coupled GPCR. On this conceptual background, the study of the Xiao group published in this issue of *Molecular Pharmacology* adds an additional layer of complexity to the topic of functional selectivity. Together with the light receptor, rhodopsin, the β_2AR is the best-studied GPCR in terms of functional domains, ligand-receptor interactions, G-protein and effector coupling, physiological function (Gether and Kobilka, 1998; Rohrer and Kobilka, 1998) and high-resolution crystal structures (Cherezov et al., 2007; Rosenbaum et al., 2007). The catecholamines (-)-epinephrine, a hormone produced

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by the adrenal medulla, and (-)-norepinephrine, a neurotransmitter, are the endogenous ligands of the β_2AR. The β_2AR is a classic G_s-coupled GPCR, triggering activation of adenylyl cyclase with subsequent cAMP production. In 1995, Xiao et al. made the most intriguing observation that pertussis toxin, ADP-ribosylating G_i-protein α-subunits and, thereby, uncoupling GPCRs from G_i, enhanced β_2AR-mediated positive inotropic effects in isolated rat cardiomyocytes. These data suggested that in addition to G_s, the β_2AR also couples to G_i-proteins in native systems. Then, in 2003, Xiao et al. noted that the positive inotropic responses towards most β_2AR agonists such as zinterol, salbutamol (albuterol) and procaterol were enhanced by pertussis toxin pretreatment, whereas the effects of fenoterol were not affected. These findings were the first indication that fenoterol may stabilize a unique β_2AR conformation promoting a distinct pattern of G-protein activation.

Different efficacies of GPCR ligand stereoisomers. It has been known for a century that the naturally occurring (-)-stereoisomer of epinephrine constricts blood vessels more potently than (+)-epinephrine (Cushny, 1908). Since then, a large number of studies has elaborated the concept that (-)-stereoisomers of catecholamines bind to adrenergic receptors with higher affinity and activate the receptors with higher potency than the corresponding (+)-stereoisomers. This concept has recently been covered in an excellent review (Patil et al., 2008).

Intriguingly, some studies did not only report differences in affinity/potency between stereoisomers but also differences in efficacy. Specifically, (-)-norepinephrine induces a slow conformational change in purified β_2AR, whereas (+)-norepinephrine is inactive in this respect (Swaminath et al., 2004). Additionally, in the presence of inosine-5'-triphosphate or xanthosine-5'-triphosphate, but not in the

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presence of guanosine-5'-triphosphate (GTP), (+)-isoproterenol is less efficacious than (-)-isoproterenol at activating adenylyl cyclase by a β_2AR-$G_{s\alpha}$ fusion protein (Seifert et al., 1999). There are also striking differences in the efficacies of (-)- and (+)-isoproterenol at stabilizing high-affinity agonist binding, i.e. ternary complex formation, in a constitutively activate β_2AR mutant (β_2AR_{CAM}) (Seifert et al., 2001). Moreover, differences in efficacy between (-)- and (+)-epinephrine were observed in native tissues and cell membranes (Kim et al., 1981; Patil et al., 1996). Beyond catecholamines, *dex*medetomidine is a partial α_{2A}-adrenergic receptor agonist, whereas the other stereoisomer, l*evo*medetomidine, acts as inverse agonist, i.e. a ligand that stabilizes the inactive (R) state of the GPCR (Jansson et al., 1998). Furthermore, stereoisomers of chiraprodifens, a new class of potent synthetic histamine H_1-receptor agonists, exhibit striking species-specific pharmacological properties at the human, rat, guinea big and bovine H_1-receptor (Strasser et al., 2008). Collectively, all these data provide the basis for the concept that stereoisomers of a given ligand stabilize functionally distinct GPCR conformations.

Differential G_s- and G_i-protein activation by fenoterol stereoisomers. The analysis of the unique effects of fenoterol in cardiomyocytes was complicated by the fact that the compound possesses two chirality centers, yielding four fenoterol stereoisomers, referred to as *R,R*-, *R,S*- *S,R*-, and *S,S*-fenoterol (Jozwiak et al., 2007). The study of Woo et al. focused on the analysis of *R,R*- and *S,R*-fenoterol, and the fenoterol derivatives, *R,R*-methoxyfenoterol and *S,R*-methoxyfenoterol in isolated rat cardiomyocytes. The compounds studied exhibit selectivity for the β_2AR relative to the β_1AR, and the *R,R*-stereoisomers possess considerably higher affinity for the β_2AR than the *S,R*-stereoisomers (Jozwiak et al., 2007). *R,R*- and *S,R*-

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fenoterol are similarly efficacious at increasing contractility of the cardiomyocytes via the β_2AR. Most strikingly, pertussis toxin shifts the contraction concentration-response curve for *S,R*-fenoterol to the left, whereas the stimulatory effects of *R,R*-fenoterol are unaffected by the toxin. Moreover, *S,R*-fenoterol stimulates extracellular signal-regulated kinase (ERK) phosphorylation in a pertussis toxin-sensitive fashion, whereas the stimulatory effects of the corresponding *R,R*-stereoisomers are pertussis toxin-insensitive. These data indicate that *R,R*-fenoterol activates only G_s-proteins, whereas and *S,R*-fenoterol activates both G_i and G_s (Fig. 1). This interpretation is supported by G-protein photoaffinity labeling experiments. Specifically, *R,R*-fenoterol is more effective at stimulating the incorporation of [γ-^{32}P]GTP azidoanilide into $G_{s\alpha}$ than *S,R*-fenoterol, whereas only *S,R*-fenoterol stimulates photoaffinity labeling of $G_{i\alpha 2}$. $G_{i\alpha 2}$ is the predominant pertussis toxin-sensitive G-protein in the heart. Thus, the previously noted PTX-insensitivity of the effects of racemic fenoterol in cardiomyocytes (Xiao et al., 2003) could be explained by the fact that the higher potency of *R,R*-fenoterol at the β_2AR for G_s masked the effect of *S,R*-fenoterol, possessing a lower potency for G_s and G_i (Fig. 1). These data show that it is sometimes misleading to use the evaluative terms eutomer and distomer (introduced by E. J. Ariëns for the more and less potent stereoisomer, respectively) which are by definition based on a particular affinity and/or potency. However, the distomer may actually possess more interesting and important pharmacological properties than the eutomer (Fig. 1).

There are some intriguing pharmacological differences in the effects of the *R,R*- and *S,R*-fenoterol pair relative to the *R,R*- and *S,R*-methoxyfenoterol pair in cardiomyocytes. Specifically, *R,R*- and *S,R*-methoxyfenoterol are similarly less efficient at activating $G_{s\alpha}$ compared to *R,R*-fenoterol and do also not significantly couple to $G_{i\alpha 2}$. Moreover, *R,R*- and *S,R*-methoxyfenoterol are more efficacious at

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activating $G_{i\alpha 3}$ compared to the corresponding *R,R*- and *S,R*-fenoterol stereoisomers. Pertussis toxin does only slightly shift the contraction concentration-response curve for *R,R*-methoxyfenoterol to the left since coupling to $G_{s\alpha}$ predominates coupling to $G_{i\alpha 2}$. This is not the case for both *S,R*-derivatives, which, therefore, show distinct pertussis toxin sensitivity of the contractility. Thus, ligand stereoisomers may possess complex structure/activity relationships with respect to efficacy of G-protein activation that cannot readily be deduced from routine receptor affinity measurements (Jozwiak et al., 2007).

Collectively, the major contribution of the work by Woo et al. is to demonstrate ligand stereoisomer-specific activation of various G-protein isoforms that most likely results from stereoisomer-specific GPCR conformations. It is important to note that Woo et al. conducted their studies with isolated rat cardiomyocytes, i.e. a cell system that can be considered as physiologically highly relevant. In support of the concept developed in this study are previous data from our laboratory using a recombinant expression system. Particularly, the potencies and efficacies of a series of “standard” experimental β_2AR (partial) agonists ((-)-isoproterenol, salbutamol, dobutamine, (-)-ephedrine and dichloroisoproterenol in order of decreasing efficacy) at β_2AR-$G_{i\alpha}$ fusion proteins differ quite substantially from the corresponding parameters at β_2AR-$G_{s\alpha}$ fusion proteins as assessed in the [^{35}S]guanosine-5’-[γ-thio]triphosphate binding assay (Wenzel-Seifert and Seifert, 2000). However, in our previous study we missed the unique opportunity to dissect differences in G-protein coupling of ligand stereoisomers because we deemed this possibility as too unlikely, trying to “focus” our study on the supposedly “important” ligands. This is an excellent example of how wrong a priori probability assumptions can delay scientific progress for several years.

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How does the β_2AR activate G_i-proteins? In addition to making an important contribution to our understanding of the molecular mechanisms of GPCR activation, the study of Woo et al. yielded an unexpected novel approach to address the controversial and very important issue of how βARs activate G_i-proteins. Previous reconstitution studies with purified human β_2AR and turkey βAR showed that βARs can activate G_i in terms of guanine nucleotide exchange to some extent without the apparent need for additional proteins and phosphorylation reactions (Asano et al., 1984; Cerione et al., 1985). In agreement with these data, the analysis of β_2AR co-expressed with $G_{i\alpha2}$ and of a β_2AR-$G_{i\alpha2}$ fusion protein in Sf9 insect cell membranes revealed that the β_2AR activates $G_{i\alpha2}$ more sluggishly than classic G_i-coupled receptors such as the formyl peptide receptor (Wenzel-Seifert and Seifert, 2000). In contrast, an alternative hypothesis had proposed that β_2AR-G_i-coupling depends on protein kinase A-mediated β_2AR phosphorylation (Daaka et al., 1997; Zamah et al., 2002). However, analysis of a β_2AR mutant devoid of protein kinase A phosphorylation sites did not support the latter hypothesis (Friedman et al., 2002). In their present study, Woo et al. found that all four fenoterol stereoisomers are similarly efficacious at stimulating protein kinase A-mediated β_2AR phosphorylation. The similar effects of stereoisomers on phosphorylation are in marked contrast to the differential effects of stereoisomers on activation of $G_{s\alpha}$, $G_{i\alpha2}$ and $G_{i\alpha3}$ as assessed by photoaffinity labeling. In unpublished studies performed by one of us (R.S.), preincubation of β_2AR-$G_{i\alpha2}$-expressing Sf9 membranes with the catalytic subunit of protein kinase A, ATP and Mg^{2+} had no effect on the efficiency of GPCR/G-protein coupling. In aggregate, the available data indicate that protein kinase A-mediated phosphorylation is not a conditio sine qua non for β_2AR/G_i coupling to occur.

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Interaction of fenoterol stereoisomers with the β_2AR at the molecular level. Although the study by Woo constitutes a major advance in the field of GPCR conformations, several important questions remain to be solved. Most importantly, how do the assumed *R,R*- and *S,R*-fenoterol-specific β_2AR conformations look like at the molecular level? The ultimate answer to this question will have to come from crystal structures of different states of the β_2AR bound to fenoterol stereoisomers. However, so far, only the crystal structure of the β_2AR bound to an inverse agonist, carazolol, could be resolved (Rosenbaum et al., 2007; Cherezov et al., 2007)). Given the high conformational flexibility and instability of the β_2AR, it will be a formidable challenge to obtain high-resolution β_2AR crystals bound to fenoterol. Perhaps this goal can be accomplished by generating β_2AR-G_α(peptide) crystals as has been very recently shown for rhodopsin (Scheerer et al., 2008). It is certainly feasible to obtain insights into the mechanism of action of fenoterol stereoisomers by studying all steps of the G-protein cycle with β_2AR-G_α fusion proteins (Wenzel-Seifert and Seifert, 2000; Weitl and Seifert, 2008) at high sensitivity and to conduct fluorescence spectroscopy studies with purified β_2AR (Swaminath et al., 2004). In addition, molecular modeling of the β_2AR ligand binding site based on the available crystal structures is possible (Audet and Bouvier, 2008).

Although the high-resolution β_2AR crystal structure probably represents an inactive receptor state, docking of the fenoterol stereoisomers may provide suggestions about possible reasons for functional selectivity. Figure 2 shows the minimized complexes of the β_2AR with *R,R*- and *S,R*-fenoterol, respectively. Both isomers are docked in a favorable extended conformation close to an energy minimum. Assuming that the general binding mode corresponds to that of carazolol and that, in particular, the projection of the *N*-isopropyl and the *N*-1-methyl-2-

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phenylethyl substituent is similar, the β_2AR selectivity of the fenoterol and methoxyfenoterol isomers may be based on an H bond of the oxygen in *para* position with the imidazolyl-NH of His-93 in TM2 (β_1AR: Ile). However, the *para* hydroxy group of the fenoterol isomers can form a second H bond with the backbone of Cys-191 in E2. This may account for the higher potency of *R,R*-fenoterol compared to *R,R*-methoxyfenoterol. The *p*-hydroxyphenyl ring of both fenoterol isomers is perpendicularly arranged with respect to the indole moiety of Trp-109 (TM3). Back-folding of the aralkyl group onto Tyr-308 (TM7) like suggested from CoMFA results (Jozwiak et al., 2007) and modeling (Audet and Bouvier, 2008, for bucindolol) is not likely in the case of the fenoterol derivatives due to sterical hindrance in the new β_2AR structure. The models in Figure 2 are also consistent with the stereoselectivity at the C1 atom of *N*-1-methyl-2-phenylethyl substituted derivatives (Jozwiak et al., 2007) since, in the eutomeric *R* configuration, the methyl group may form direct van der Waals contacts with the side chains of Trp-109 and Thr-110 in TM3.

In the case of *R,R*-fenoterol, the ethanolamine moiety is involved in a distinct network of hydrogen bonds and ionic interactions with the β_2AR (Fig. 2A). The protonated amine forms a salt bridge with Asp-113 (TM3) and a charge-assisted H bond with the side chain oxygen of Asn-312 (TM7). The hydroxy group may interact with the β_2AR via two H bonds, one as donor for Asp-113 (charge-assisted), and another one as acceptor for the side chain amide of Asn-312. The phenylethyl moiety closely fits into a hydrophobic pocket consisting of Val-114 and Val-117 (TM3), Phe-193 (E2), as well as Trp-286, Phe-289 and Phe-290 (TM6). Rotameric changes of Trp-286 are believed to induce the "toggle switch" for receptor activation (Schwartz et al., 2006). The *meta* hydroxy groups form H bonds with Ser-204 and Ser-207,

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respectively, in TM5. Both serines are critical for catecholamine binding and β_2AR activation (Strader et al., 1989).

In summary, the model in Figure 2A suggests a very tight binding of *R,R*-fenoterol to the β_2AR which is in particular based on seven hydrogen bonds. *S,R*-fenoterol can principally bind in a similar mode and conformation (see Figure 2B). However, the interactions with the β_2AR are weaker compared to the *R,R*-stereoisomer since the orientation of the OH group does not enable the H bonds with Asp-113 and the amide NH_2 of Asn-312 (instead, an H bond with the side chain oxygen of this asparagine is possible). In the distomeric *S* configuration, the hydroxy group is projected onto the phenyl ring of Phe-289. One *meta* OH substituent forms an H bond with Ser-203 in place of that of the *R,R*-isomer with Ser-204. The minimization has shifted the phenethanolamine moiety of *S,R*-fenoterol by up to 1 Å compared to its position in the β_2AR-*R,R*-fenoterol complex, indicating greater flexibility due to lacking interactions. This phenomenon should be further analyzed by molecular dynamics simulations. To suggest a completely different binding mode of both fenoterol isomers, e.g., a reverse fit of the phenyl moieties in the case of the *S,R*-derivative, would be rather bold since the stereoselectivity of the second chiral center is generally the same independent of the configuration of the OH group.

Taken together, the models indicate possible reasons for the functional selectivity of fenoterol and methoxyfenoterol stereoisomers. It appears that deviations from the "normal" predominant G_s coupling of the β_2AR and more promiscuity with respect to different G protein species just occur if certain interactions cannot be formed. Then the ligand-receptor complex has more degrees of conformational freedom than in the case of a tightly bound structure (like *R,R*-fenoterol). The higher flexibility enables the generation of a greater number of alternative conformations from which some may indeed represent "ligand-specific active receptor states"

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interacting more or less selectively with $G_{s\alpha}$, $G_{i\alpha 2}$, or $G_{i\alpha 3}$. I.e., ligand-specific inactive GPCR conformations are no necessary condition for promiscuous G protein coupling. This hypothesis must be further substantiated by molecular dynamics simulations and can be verified only by crystal structures of active GPCR states. A first milestone on this long way has been very recently set by the release of an opsin structure in its G-protein-interacting conformation (Scheerer et al., 2008), showing an outward tilt of TM6 and a pairing of TMs 5 and 6.

Some future studies. *R,R*-Fenoterol is a G_s-selective full β_2AR agonist, whereas *S,R*-fenoterol is a partial β_2AR agonist with respect to G_s-activation and a full agonist with respect to $G_{i\alpha 2}$-activation (Fig. 1). It will be important to develop G_i-selective β_2AR agonists in order to learn more about the as yet elusive (patho)physiological relevance of the β_2AR-activated G_i pathway in cardiomyocytes and other systems such as bronchial smooth muscle cells. This will not be trivial since to this end, the potency of most β_2AR agonists for the G_i pathway is considerably lower than for the G_s pathway (Wenzel-Seifert and Seifert, 2000). Moreover, high potency of G_i-selective β_2AR agonists would be most welcome in order to avoid potential ligand interactions with the β_1AR and β_3AR. Future studies will also have to address the question whether in addition to G_s and G_i, fenoterol stereoisomers differentially activate pertussis toxin-sensitive G_q-proteins that mediate phospholipase C activation. The necessity for such studies comes from the finding that the stimulatory effects of the four fenoterol stereoisomers examined exhibit striking differential and partial pertussis toxin-insensitivity which is not discussed in the paper by Woo et al., however. Previous studies from our laboratory had already shown that the pharmacological profile of the β_2AR coupled to G_q-proteins differs

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from the profile of the G_s- and G_i-coupled β_2AR, although G_q-coupling was rather poor in our hands (Wenzel-Seifert and Seifert, 2000). It will also be necessary to study the impact of G-protein $\beta_x\gamma_y$-complexes on G_α-selectivity of fenoterol stereoisomers. Particularly, the stereosiomers differ remarkably from each other in terms of $G_{s\alpha}$ activation, but in terms of β_2AR phosphorylation, they are quite similar. An explanation for this discrepancy could be the recruitment of different $G\beta_x\gamma_y$ -complexes by the various stereoisomers, compensating for the differences observed with respect to $G_{s\alpha}$ activation.

The current study by Woo et al. focuses on efficacies of fenoterol stereoisomers with regard to the various parameters. While this is sufficient to support the main hypothesis of the paper, future studies should also careful examine agonist potencies since it is well possible that a given ligand does not only possess multiple efficacies but also multiple affinities for the various receptor conformations, depending on the specific parameter analyzed. These studies will bring us a step closer to the goal of achieving ligand-specific activation of G_α-protein subtypes. Multiple parameter-dependent affinities/potencies of a given ligand for the β_2AR have been observed before (Seifert et al., 1999, 2001; Weitl and Seifert, 2008).

Elegant studies with opioid receptors have shown that various ligands differ from each other in their membrane and cell compartment trafficking (Hanyaloglu and von Zastrow, 2008). Thus, it is conceivable that fenoterol stereoisomers show differences in β_2AR trafficking as well. Specifically, resistance of fenoterol/β_2AR complexes to desensitization and internalization may occur since long-term treatment with fenoterol in a heart failure model is not associated with a loss of efficacy (Ahmet et al., 2008). In this context, it will also be interesting to examine the effects of

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fenoterol stereoisomers on long-term effects such as gene expression using the microarray technique.

The study by Woo et al. has important implications for future agonist screening programs in the pharmaceutical industry. It is clear that any drug development program dealing with stereoisomers should not only search for “more active” high-potency eutomers, but also for “less active” low-potency distomers (Fig. 1). Moreover, it is prudent to study at least two independent read-outs for each GPCR, preferably mediated by different G-proteins. And even for GPCRs that couple only to one cognate G-protein, it is advisable to determine several read-outs at various steps of the G-protein cycle since differences of ligand potencies and efficacies for the various parameters within the cycle can be substantial, even if endogenous agonists are considered (Seifert et al., 1999, 2001; Weitl and Seifert, 2008). On first glance, this may sound like bad news since initially, a drug development program will become more complicated, expensive and time-consuming. However, on second glance, the return may be novel interesting drugs that would have been missed using standard approaches aiming only at high-potency ligands and measuring only one single read-out.. We have missed such a great opportunity in our laboratory several years ago (Wenzel-Seifert and Seifert, 2000).

Clinical implications. The present study has important clinical implications. Specifically, fenoterol is widely used for the treatment of acute asthma attacks, and uncritical use of racemic fenoterol is associated with increased mortality of asthma patients (Jalba, 2008). Thus, clinical studies will have to answer the question whether specific fenoterol stereoisomers, through their unique pattern of G-protein activation, possess clinically relevant differences in terms of therapeutic efficacy and toxicity. Along the same line, albuterol possesses a chirality center (Boulton and Fawcett,

2002; Broadley, 2006). However, to this end, it is unclear whether *lev*albuterol exhibits any relevant clinical advantages compared to racemic albuterol. While additional clinical studies on this topic are certainly warranted, it is also important to characterize *lev*albuterol and *dex*albuterol in detail at the molecular and cellular level since so far, most mechanistic studies have been performed only with racemic albuterol (Seifert et al., 1999, 2001; Wenzel-Seifert and Seifert, 2000). The reason for this situation is simply the limited stereoisomer availability to the pharmacological community.

Another potential application of β_2AR agonists is the treatment of heart failure as adjunct to β_1AR antagonists. Specifically, in a rat model of dilated cardiomyopathy, long-term treatment with racemic fenoterol enhances the beneficial effects of β_1AR blockade with metoprolol (Ahmet et al., 2008). Again, it will be interesting to examine what the effects of fenoterol stereoisomers in this model are. The efficacy of the agonist in this long-term treatment setting points to the lack of relevant desensitization.

Finally, we have recently shown that the endogenous β_2AR agonists (-)-epinephrine and (-)-norepinephrine interact differentially with the β_2AR and β_1AR coupled to either of the two $G_{s\alpha}$ splice variants (Weitl and Seifert, 2008). Both catecholamines are used clinically in life-threatening conditions such as cardiac arrest and septic shock. The careful analysis of the corresponding (+)-enantiomers of catecholamines may yield drugs with improved clinical properties relative to the naturally occurring (-)-enantiomers. This possibility has not yet been explored at all. In this context, it will also be very interesting and important to determine whether fenoterol stereoisomers interact differentially with the various human β_2AR polymorphic isoforms (Brodde, 2008).

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Conclusions. The current study by the Xiao group corroborates the concept of ligand-specific GPCR conformations, resulting in differential G-protein activation or, in more general terms, functional selectivity. Ligand stereoisomers are important experimental tools to examine mechanisms of GPCR activation and signal transduction pathways. The present study should encourage pharmacologists to systematically examine the “more active” eutomer and the “less active” distomer of a given ligand and not only to focus on more readily available racemic ligands or “eutomers”. We anticipate that analysis of ligand stereoisomers with modern pharmacological and biophysical methods will be a goldmine and yield important data, ultimately improving clinical drug therapy and reducing drug toxicity.

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Footnotes

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Figure legends

Fig. 1. Differential activation of the β_2AR, G-proteins and signal transduction pathways by *R,R*-fenoterol and *S,R*-fenoterol in cardiomyocytes. The scheme was developed on the basis of the data by Woo et al. Please note that *R,R*-methoxyfenoterol and *S,R*-methoxyfenoterol exhibit different G-protein specificity than the corresponding fenoterol stereoisomers. AC, adenylyl cyclase; ERK extracellular signal-regulated kinase; PTX, pertussis toxin.

Fig. 2. Model of the interaction of *R,R*-fenoterol and *S,R*-fenoterol with the β_2AR based on the β_2AR crystal structure (PDB 2RH1). The ligands were manually docked and the complexes were in turn minimized with the Amber-FF99 (β_2AR with fixed ligand) and the Tripos force field (ligands and a "hot" receptor region of amino acids up to 6 Å distant). Modeling was performed with Sybyl 7.3 (Tripos, L.P., St. Louis). Both panels show the side chains and Cα atoms of all amino acids within 3 Å around the ligands (additionally, Tyr-308 and a backbone sequence in E2) as sticks and the Cα trace of the TM regions as lines. The backbone, the carbon and some essential hydrogen atoms of the amino acids are individually drawn in spectral colors: TM2-orange, TM3-yellow, E2-cyan, TM5-greenblue, TM6-blue, TM7-purple. All nitrogens – blue, oxygens – red, sulfur - yellow. Atoms of the β_2AR suggested to be involved in H bonds with the ligand are marked as balls. (**A**) Docking of *R,R*-fenoterol (ball and stick model with grey carbon and essential hydrogen atoms). (**B**) Docking of *S,R*-fenoterol (ball and stick model with pink carbon and essential hydrogen atoms). For comparison, the scaffold of *R,R*-fenoterol (stick model, grey C atoms) from panel A is additionally drawn, based on a superposition of all binding site atoms of both models (RMS deviation 0.47 Å).

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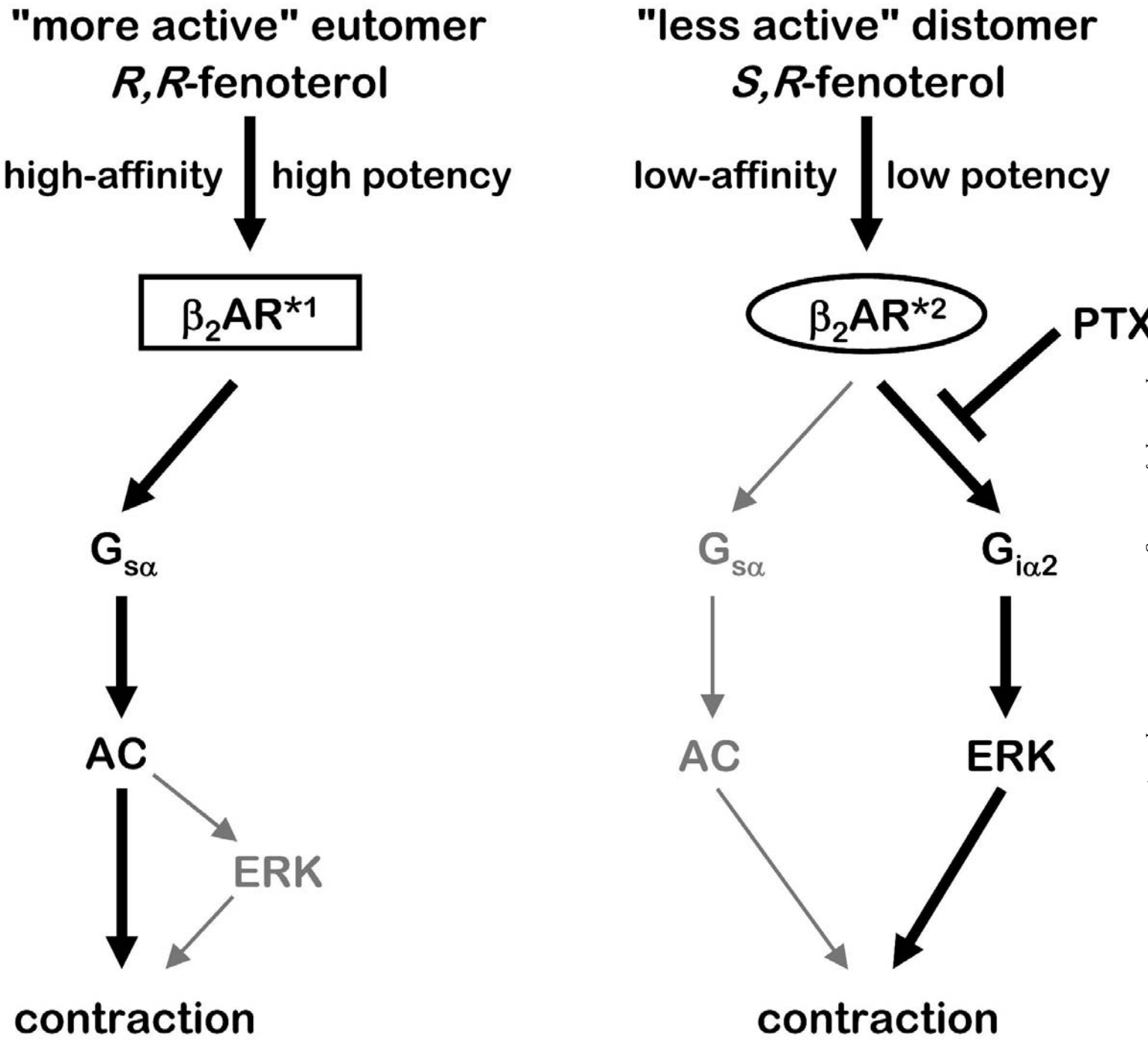

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