Hierarchy of Polymorphic Variation and Desensitization Permutations

Relative to β₁- and β₂-adrenergic Receptor Signaling*

Deborah A. Rathz‡, Kimberly N. Gregory‡, Ying Fang‡, Kari M. Brown§ and Stephen B. Liggett‡§¶

Departments of ‡Pharmacology, and §Medicine

University of Cincinnati College of Medicine, Cincinnati, Ohio 45267

Running Title: β₁-adrenoceptor Genotype and Desensitization

*Supported by NIH grants HL22619, HL52318, GM61376, and HD07463

Corresponding author:
Stephen B. Liggett
University of Cincinnati College of Medicine
231 Albert Sabin Way, Room G-062
Cincinnati, OH 45267-0564
Telephone: 513-558-4831
FAX: 513-558-0835
E-mail: stephen.liggett@uc.edu
Summary

Agonist-promoted desensitization of G-protein coupled receptors results in partial uncoupling of receptor from cognate G-protein, a process which provides for rapid adaptation to the signaling environment. This property plays important roles in physiologic and pathologic processes as well as therapeutic efficacy. However, coupling is also influenced by polymorphic variation, but the relative impact of these two mechanisms on signal transduction is not known. To determine this we utilized recombinant cells expressing the human β1AR or a gain-of-function polymorphic variant (β1AR-Arg389), and the β2AR or a loss-of-function polymorphic receptor (β2AR-Ile164). Adenylyl cyclase activities were determined with multiple permutations of the possible states of the receptor: genotype, basal or agonist stimulated, and with or without agonist pre-exposure. For the β1AR, the enhanced function of the Arg389 receptor underwent less agonist-promoted desensitization compared to its allelic counterpart. Indeed, the effect of polymorphic variation on absolute adenylyl cyclase activities was such that desensitized β1AR-Arg389 signaling was equivalent to non-desensitized wild-type β1AR; that is, the genetic component had as much impact as desensitization on receptor coupling. In contrast, the enhanced signaling of wild-type β2AR underwent less desensitization compared to β2AR-Ile164, thus the heterogeneity in absolute signaling was markedly broadened by this polymorphism. Inverse agonist function was not affected by polymorphisms of either subtype. A general model is proposed whereby up to 10 levels of signaling by G-protein coupled receptors can be present based on the influences of desensitization and genetic variation on coupling.
Introduction

Like a number of other G-protein coupled receptors, the $\beta_1$- and $\beta_2$- adrenergic receptor ($\beta_1$AR, $\beta_2$AR)\(^1\) undergo desensitization during continuous exposure to agonist. Such desensitization occurs maximally after several minutes of agonist exposure and is due to decreased interaction with G\(_s\), which is evoked by receptor phosphorylation (1). Thus the signal transduction of these receptors can be characterized as one of two potential conditions or states, defined here as “control” (no recent exposure to agonist) and “desensitized”. However, we have recently shown that an alteration in receptor-G\(_s\) coupling can also be imposed by genetic mechanisms. A single nucleotide polymorphism found in the $\beta_1$AR gene in the human population (2, 3) results in either Gly or Arg being encoded at amino acid position 389 of the proximal portion of the cytoplasmic tail. In studies using transfected cells with equivalent expression of the two receptors, the $\beta_1$AR-Arg389 displays an increase in G\(_s\) coupling compared to $\beta_1$AR-Gly389 (2). So, one can consider that the human $\beta_1$AR can exist in four agonist-stimulated states: Gly389 control and desensitized, and Arg389 control and desensitized. And, since basal (non-agonist) activity is also affected by these genetic and desensitization processes, eight states can be considered. For the $\beta_2$AR, a polymorphism which results in a substitution of Ile for Thr at amino acid 164 in the fourth transmembrane spanning domain results in a decrease in G\(_s\) coupling (4). Thus, eight such states can be considered for the $\beta_2$ARs as well, again based on genotype and desensitization status. As opposed to their allelic variants, only the $\beta_1$AR-Gly389 and the $\beta_2$AR-Thr164 (the receptors often designated as “wild-type”) have been studied in regards to desensitization in recombinantly expressing cells (5, 6). Based on the significant impact that both desensitization and polymorphic variation have on coupling, we considered that control signaling with one variant might even be equivalent to the desensitized signaling of the other. Since inverse
agonists act to lower the frequency of spontaneous activation of βARs, there is the potential for coupling polymorphisms to influence this response as well. Knowing the hierarchy of these states facilitates understanding the molecular basis of receptor response to therapeutic agents and receptor dysfunction that can occur in pathologic states, where both genetic and post-translational modifications occur together. Such studies also provide for a general model which depicts the interaction of genetic and desensitization mechanisms in G protein coupled receptor signaling. To investigate this, we expressed these four receptors in Chinese hamster fibroblasts at equivalent levels and studied the relative effects of these genetic modifications and those of short-term agonist-promoted desensitization on receptor function.
**Experimental Procedures**

*Constructs and transfections*

Site-directed mutagenesis was performed on the wild-type cDNA templates as previously described so as to mimic the human Arg389 β1AR and Ile164 β2AR variants (2, 4). Wild-type and polymorphic cDNAs were cloned into the mammalian expression vector pBC12B1. CHW-1102 cells were stably transfected by calcium phosphate precipitation. Positive clones were selected based on resistance to 300 μg/ml G418. Cultures were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum at 37°C, 5% CO2, in 100 μg/ml streptomycin, 100 units/ml penicillin, and 80 μg/ml G418. COS-7 cells were transiently transfected and maintained as described (7).

*Radioligand Binding*

Confluent layers of CHW cells were washed three times with cold phosphate buffered saline (PBS), lysed in hypotonic buffer (5 mM Tris, 2 mM EDTA, pH 7.4) and mechanically detached with a rubber policeman in a small volume. The particulates were homogenized with a polytron and then centrifuged at 42,000 x g for ten minutes. Pellets were then resuspended in 75 mM Tris, 12.5 mM MgCl2, 2mM EDTA, pH 7.4. Expression levels were determined in saturation binding assays. Membranes were incubated with 400 pM 125I-cyanopindolol (125I-CYP) and 100 μM GTP for 2 hours at room temperature with nonspecific binding determined in the presence of 1 μM propranolol. Reactions were stopped by dilution and vacuum filtration over Whatmann glass fiber filters. The percentage of the receptor pool that is expressed at the cell surface was determined exactly as described (8). Briefly, cells were homogenized as above, centrifuged at 400 x g for 10 min, and the supernatant layered over a 35% sucrose cushion and centrifuged at
150,000 x g for 1.5 hrs. The 0-35% interface (light vesicular membranes) and the pellet (plasma membranes) were collected, diluted in 5 mM Tris, 2 mM EDTA, pH 7.4 and centrifuged at 200,000 x g for 1 hr. Radioligand binding with $^{125}$I-CYP was then carried out with each fraction as described above.

**Adenylyl cyclase activities, cAMP measurements and desensitization protocol**

Confluent monolayers of cells were washed twice with Hank’s balanced salt solution (HBSS) and allowed to equilibrate in fresh DMEM for 30 minutes at 37°C, 5% CO$_2$. Cells were then incubated with either 10 µM of the indicated agonist with 100 µM ascorbic acid or with ascorbic acid alone (control) for 20 minutes, washed five times with cold PBS, detached, and membranes prepared as above. Membranes were incubated with 30 mM Tris, pH 7.4, 2mM MgCl$_2$, 0.8 mM EDTA, 120 µM ATP, 60 µM GTP, 100 µM cAMP, 2.8 mM phosphoenolpyruvate, 2.2 µg myokinase, the indicated concentrations of agonist, and 1 µCi [$\alpha$-$^{32}$P]ATP for 30 minutes at 37°C as previously described (2). The stop buffer contained a [$^3$H]cAMP standard, which accounted for column recovery. [$^{32}$P]cAMP was separated from [$\alpha$-$^{32}$P]ATP chromatographically using alumina columns. Untreated (control) cells bearing the two β$_1$ARs are designated Arg389$^c$ and Gly389$^c$, while those studied after treatment with 10 µM of the agonist epinephrine (desensitized) are designated Arg389$^d$ and Gly389$^d$. Similarly, control β$_2$AR are designated Thr164$^c$ and Ile164$^c$ while the desensitized state has the subscript "D". An additional qualifier, based on whether the adenylyl cyclase response is in the absence of agonist (basal, B) or in response to isoproterenol (I), provides for eight different permutations. To ascertain the effects of inverse agonists, COS-7 cells were transfected with the indicated receptors and G$\alpha$s. At confluence, cells in 24 well plates were washed and incubated with 100
μM IBMX with or without varying concentrations of inverse agonists for 45 min. cAMP produced over this time was quantitated by a competitive immunoassay (Amersham).

**Miscellaneous**

Protein concentrations were determined by the copper bicinchoninic acid method (9). Curve fitting was carried out with PRISM software (GraphPad, San Diego, CA). Dose-response curves were compared by ANOVA with post-hoc t-tests when the p value was <0.05. Other results were compared with paired t-tests as indicated. Data are presented as mean ± standard error.
Results

Expression levels of the two $\beta_1$AR variants in the membrane preparations utilized for the adenylyl cyclase assays were $206 \pm 16$ for Gly389 and $170 \pm 22$ fmol/mg for Arg389. Likewise, the two $\beta_2$AR cell lines had similar levels of expression (Thr164=783 $\pm$ 88, Ile164=1104 $\pm$ 111 fmol/mg). Of note, signaling characteristics were compared between the two $\beta_1$AR variants, but not between subtypes. There was no evidence for relevant intracellular accumulation of either polymorphic variant as determined by radioligand binding of light vesicular membrane and plasma membrane fractions (table 1). Our initial goal was to assess the degree of agonist-promoted desensitization for the wild-type $\beta_1$AR (Gly389) and the Arg389 polymorphic receptor, and the wild-type $\beta_2$AR (Thr164) and its variant, Ile164. Concerning the $\beta_1$ARs, we knew from previous studies that basal and agonist-stimulated adenylyl cyclase activities (in absolute values) were higher for the Arg389 $\beta_1$AR compared to the Gly389 receptor. For the current work, cells in culture were exposed to vehicle or agonist for 20 min, washed, membranes prepared, and adenylyl cyclase activities determined. When desensitization is quantitated as the percent decrease of the response relative to that in the absence of agonist pretreatment, the $\beta_1$AR-Gly389 underwent $21\pm6.7\%$ agonist-promoted desensitization (fig 1a, table 1). No change in the EC$_{50}$ was observed. The hyperfunctional $\beta_1$AR-Arg389 underwent a greater degree of desensitization compared to its allelic variant, amounting to $34\pm4.1\%$ desensitization ($p<0.01$ vs $\beta_1$AR-Gly389, fig 1b). For the $\beta_2$ARs, we also found that the two polymorphic variants differed in the extent of agonist-promoted desensitization (fig 1c, 1d). However, in contrast to what was observed with the $\beta_1$AR, the hyperfunctional $\beta_2$AR-Thr164 actually underwent decreased desensitization. $\beta_2$AR-Thr164 displayed $26\pm4.0\%$ desensitization vs $37\pm4.6\%$ found for Ile164-$\beta_2$AR ($p <0.05$).
Although the above data examine the extent of desensitization as a percentage of the control response, the absolute levels of adenylyl cyclase activities (pmol/min/mg) establish a hierarchy of signal transduction based on genotype and desensitization. For the \( \beta_1 \)AR, these data are shown in figure 2a. As is seen, the influence of genetic variation was such that even after desensitization, the maximal Arg389 receptor function (Arg\( _{\text{DI}} \)) was equivalent to the maximal non-desensitized Gly389 variant (Gly\( _{\text{CI}} \)). The rank order of activities for the various states for the \( \beta_1 \)AR are: \( \text{Arg}_{\text{CI}} > \text{Gly}_{\text{CI}} = \text{Arg}_{\text{DI}} > \text{Arg}_{\text{CB}} > \text{Gly}_{\text{CB}} \geq \text{Gly}_{\text{DB}} \). For the \( \beta_2 \)AR, since the genetically uncoupled Ile164 receptor underwent an even greater degree of desensitization than the wild-type (figure 1c, d), the heterogeneity in adenylyl cyclase activities due to the various permutations was substantial. The eight states are shown in figure 2b. The rank order was thus: \( \text{Thr}_{164\text{CI}} > \text{Thr}_{164\text{DI}} > \text{Ile}_{164\text{CI}} > \text{Ile}_{164\text{DI}} > \text{Thr}_{164\text{CB}} > \text{Thr}_{164\text{DB}} > \text{Ile}_{164\text{CB}} = \text{Ile}_{164\text{DB}} \).

We also explored whether the polymorphic variations affected the response to inverse agonists. We considered that the conformational effects of these substitutions might constrain the receptor so that it could not be fully "inactivated" (i.e., decreased spontaneous activation) by the binding of inverse agonists. We were unable to obtain consistent results in CHW cells, likely due to the low levels of basal adenylyl cyclase activity and the relatively low expression levels in the stable lines. Thus COS-7 cells were transfected with the various receptors along with \( \text{G}_{\alpha_s} \), exposed to the phosphodiesterase inhibitor IBMX, and whole cell cAMP production over the ensuing 30 minutes determined in the absence or presence of various concentrations of the inverse agonists CGP-20712 (\( \beta_1 \)AR) or ICI-118551 (\( \beta_2 \)AR). Here, it is the absolute levels of cAMP that are relevant rather than a percent change. Results are shown in figure 3. The basal levels of cAMP
were greater for β2AR Thr164 compared to β2AR Ile164, as might have been predicted from the membrane studies. Interestingly, a similar difference between the two β1ARs, which also might be expected, was not observed. This may be because spontaneous (i.e., non-agonist) activation is less apparent with β1AR (10) and thus differences in the two variants may not be as readily discerned. Regarding the responses to inverse agonists for the β1ARs, exposure to CGP-20712 resulted in a dose-dependent decrease in cAMP production for both receptors. The response was identical for both, with minimal cAMP levels of 7.1 ± 0.8 and 7.0 ± 0.9 pmol/mg for Arg389 and Gly389 (n=5). Similarly, for the β2ARs the inverse agonist ICI-118551 lowered cAMP to the same levels for both receptors (Thr164 = 7.4 ± 0.1, Ile164 = 6.5 ± 1.0 pmol/mg, n=4). These results indicate that the responses to inverse agonists are not influenced by these genetic variations.
Discussion

The dynamic nature of signaling by G-protein coupled receptors has been considered indicative of the ability of these receptors to rapidly adapt to changes in their signaling environment. Processes such as receptor desensitization are critical for maintenance of homeostasis during normal physiological circumstances, may be compensatory in certain pathological states, or may aberrantly alter signaling and contribute to characteristics of disease states (11). Desensitization may also limit the effectiveness of therapeutically administered agonists (tachyphylaxis). Using recombinantly expressed receptors, the nature of rapid agonist-promoted desensitization of many G-protein coupled receptors has been explored. Some receptors, such as the α2CAR and the β3AR, display little or no rapid desensitization (12, 13). Others display a range of desensitization attributed to various mechanisms including phosphorylation by G-protein coupled receptor kinases (GRKs), or second messenger dependent kinases such as PKA or PKC. Such comparative studies can be helpful in drug design or understanding selected features of disease states. With the recent elucidation of polymorphic variants of receptors such as the β1- and β2AR, which have significant functional impact on receptor coupling, the potential interaction between desensitization and genetic variation needs to be considered so as to establish how receptor signaling is influenced by both processes.

Here we have carried out studies with polymorphic β1ARs (2) which have either Gly or Arg at amino acid 389. This residue is located in the cytoplasmic portion of the receptor, within a predicted α helix formed between the seventh transmembrane spanning domain and the membrane anchoring palmitoylated cysteine(s) (14). Given the steric properties of Gly within α helices, and the importance of this region for G-protein binding, its not surprising that functional
coupling is different between the Arg and Gly β1AR variants. The β2AR polymorphism (4) consists of a substitution of Ile for Thr in the fourth transmembrane spanning domain, and also displays altered coupling to Gs, likely due to changes in the agonist binding pocket which affects the conformation of the intracellular loops. A priori, it was not clear whether these polymorphisms would enhance or depress agonist promoted desensitization. For example, the robust signaling of β1AR-Arg389 might render it less likely to desensitize; conversely, since its conformation is more favorable for Gs coupling, it could be more sensitive to GRK-mediated phosphorylation, which is dependent on the receptor being in the active conformation.

We show here that there is a significant impact of these polymorphisms on agonist-promoted desensitization. In the case of β1AR function, the desensitized hyperfunctional variant (Arg389) is equivalent to that of the non-desensitized Gly389 receptor. Since the basal level of signaling, which represents spontaneous conversion to R*, is also relevant, the number of permutations, taking into account basal or agonist stimulation, non-desensitized or desensitized, and two polymorphic variants, for the β1AR amounts to eight. For the β1AR this is graphically displayed in matrix format with the aforementioned states in figure 4, which is useful for considering a more generalized scheme of the role of genetic variation in G-protein coupled receptor signaling (see below). As is shown, there is considerable variation in basal and agonist-stimulated activities upon stratification by genotype and desensitization status. Such a range of relative signaling efficacy, and the complex interactions between desensitization and genotype, may explain the high degree of interindividual variability in physiologic responses to agonists and antagonists which has been observed (reviewed in (15)). An additional level of signaling can also be found when one considers the response to inverse agonists, which bind receptor and tend
to stabilize the R state, and thus minimize spontaneous conversion to R*. As such, Gs coupled receptors display a decrease in basal adenylyl cyclase activity/cAMP production. Depending on the nature of the polymorphism, the response to inverse agonist could also be affected by genetic variation. Interestingly, despite the other effects of these polymorphisms, differences in inverse agonist efficacy were not observed with either the β1AR or β2AR variants, as cAMP levels were reduced by inverse agonists to the same absolute levels regardless of genotype. (These values are not incorporated into figure 4, since they were determined using a different approach and are not directly comparable.)

For a general model (figure 5) of the potential interaction of uncoupling events due to genetic variation and to desensitization, we have made several assumptions in order to include polymorphisms with various phenotypic effects on receptor signaling. From prior characterization of agonist, receptor, and G-protein interactions of the polymorphic βAR (2, 4), we have assumed that the conformation of the receptor is altered by the polymorphisms under study. Thus the depiction of two active conformations (R$_{I}^{*1}$, R$_{II}^{*3}$) based on the two different receptors (R$_{I}$ and R$_{II}$). It is also assumed that when desensitized the conformation of the receptor is altered by phosphorylation and β-arrestin interaction (16), and these states are thus denoted as R$_{I}^{*2}$, R$_{II}^{*4}$. The "basal" (i.e., non-agonist bound) signaling of a receptor, due to spontaneous toggling to an active conformation, is also considered here as relevant, as is the effect of desensitization on basal signaling. (Although not affected with the β1AR or β2AR, the potential for a coupling polymorphism to alter inverse agonist activity is included in the general model.)

The abundance of each of the above species at equilibrium is indicated by the arrows and the subscripted brackets. Within this general model 10 relevant levels of signaling, due to the
various states or abundance of a given species, are present at steady state. Of note, other minor species of unknown signaling significance, such as agonist bound receptor that is not activated (i.e., AR₁) are not included.

From the standpoint of βAR subtypes in the heart, their functional regulation has been linked to a variety of physiological states in diseases such as heart failure. In human heart failure, myocardial β₁AR and β₂AR have been shown to be desensitized. Along with receptor downregulation, this response is thought to be adaptive in that the pathologically altered heart with limited physiologic and metabolic reserves is protected from constant stimulation by high circulating catecholamines. On the other hand, other studies in genetically altered mice have suggested that some aspects of desensitization of βAR signaling may be maladaptive in experimental heart failure (17). Recent studies have shown that β₁AR or β₂AR polymorphisms are associated with certain physiologic or pathologic phenotypes in human heart failure (18-21). However, prior to the current study it has not been clear if there was any potentiation, or attenuation, of desensitization events by these polymorphisms. The in vitro data presented here indicate that both desensitization and genetic variation together can serve to set the ultimate level of signaling of β₁AR and β₂AR. Indeed, the signaling of some receptors, even in the desensitized state, is equivalent to their non-desensitized allelic variants. Stated another way, genetic variation can have an effect of the same magnitude as that of desensitization. Regarding βAR in heart failure, this may be particularly important in defining patient subsets, tailoring therapeutic regimens, or in the development of new agents (15, 22). As a general paradigm, we present a model by which genetic variation and desensitization of G-protein coupled receptor signaling can be considered as multiple states. Although the prevalence of functional
polymorphisms within the superfamily is not fully defined (23) many G-protein coupled receptors have been reported to be polymorphic in their coding regions (24, 25), such that the model may be applicable to multiple diverse signaling events by these receptors.
References

1. Liggett, S. B. and Lefkowitz, R. J. (1993) Regulation of cellular signal transduction pathways by desensitization and amplification, John Wiley & Sons, London

2. Mason, D. A., Moore, J. D., Green, S. A., and Liggett, S. B. (1999) J.Biol.Chem. 274, 12670-12674

3. Moore, J. D., Mason, D. A., Green, S. A., Hsu, J., and Liggett, S. B. (1999) Hum.Mutat. 14, 271

4. Green, S. A., Cole, G., Jacinto, M., Innis, M., and Liggett, S. B. (1993) J.Biol.Chem. 268, 23116-23121

5. Freedman, N. J., Liggett, S. B., Drachman, D. E., Pei, G., Caron, M. G., and Lefkowitz, R. J. (1995) J.Biol.Chem. 270, 17953-17961

6. Hausdorff, W. P., Bouvier, M., O'Dowd, B. F., Irons, G. P., Caron, M. G., and Lefkowitz, R. J. (1989) J.Biol.Chem. 264, 12657-12665

7. McGraw, D. W., Forbes, S. L., Kramer, L. A., and Liggett, S. B. (1998) J.Clin.Invest. 102, 1927-1932

8. Suzuki, T., Nguyen, C. T., Nantel, F., Bonin, H., Valiquette, M., Frielle, T., and Bouvier, M. (1992) Mol.Pharmacol. 41, 542-548

9. Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D. C. (1985) Anal.Biochem 150, 76-85

10. Engelhardt, S., Grimmer, Y., Fan, G. H., and Lohse, M. J. (2001) Mol.Pharmacol. 60, 629-631
11. Bunemann, M., Lee, K. B., Pals-Rylaarsdam, R., Roseberry, A. G., and Hosey, M. M. 
   (1999) *Annu Rev Physiol* **61**, 169-192
12. Eason, M. G. and Liggett, S. B. (1992) *J.Biol.Chem.* **267**, 25473-25479
13. Liggett, S. B., Freedman, N. J., Schwinn, D. A., and Lefkowitz, R. J. (1993) 
   *Proc.Natl.Acad.Sci.U.S.A.* **90**, 3665-3669
14. Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Le 
   Trong, I., Teller, D. C., Okada, T., Stenkamp, R. E., Yamamoto, M., and Miyano, M. 
   (2000) *Science* **289**, 739-745
15. Liggett, S. B. (2001) *Nature Medicine* **7**, 281-283
16. Krupnick, J. G. and Benovic, J. L. (1998) *Annu.Rev.Pharmacol.Toxicol.* **38**, 289-319
17. Liggett, S. B. (2001) *J.Clin.Invest.* **107**, 947-948
18. Liggett, S. B., Wagoner, L. E., Craft, L. L., Hornung, R. W., Hoit, B. D., McIntosh, T. C., 
   and Walsh, R. A. (1998) *J.Clin.Invest.* **102**, 1534-1539
19. Wagoner, L. E., Craft, L. L., Singh, B., Suresh, D. P., Zengel, P. W., McGuire, N., 
   Abraham, W. T., Chenier, T. C., Dorn, G. W. II., and Liggett, S. B. (2000) *Circ.Res.* **86**, 
   834-840
20. Wagoner, L. E., Craft, L. L., Zengel, P., McGuire, N., Rathz, D. A., Dorn, G. W. I., and 
   Liggett, S. B. (2002) *Am.Heart J.* **144**, 840-846
21. Small, K. M., Wagoner, L. E., Levin, A. M., Kardia, S. L. R., and Liggett, S. B. (2002) 
   *N.Engl.J.Med.* **347**, 1135-1142
22. Liggett, S. B. (2000) *Pharmacology* **61**, 167-173
23. Small, K. M., Seman, C. A., Castator, A., Brown, K. M., and Liggett, S. B. (2002) *FEBS 
   Lett.* **516**, 253-256
24. Rana, B. K., Shiina, T., and Insel, P. A. (2001) *Annu. Rev. Pharmacol. Toxicol.* **41**, 593-624

25. Small, K. M., Tanguay, D. A., Nandabalan, K., Zhan, P., Stephens, J. C., and Liggett, S. B. (2003) *Am. J. Pharmacogenomics*
Footnote

1 The abbreviations are: βAR, β-adrenergic receptor; Gs, stimulatory guanine nucleotide binding protein; 125I-CYP, 125I-iodocyanopindolol; IBMX, isobutylmethylxanthine; C, control conditions; D, desensitized conditions; B, basal state of adenylyl cyclase activation; I, isoproterenol stimulated state of adenylyl cyclase activation.
Figure Legends

Figure 1. Agonist-promoted desensitization of polymorphic \( \beta_1 \)AR and \( \beta_2 \)AR. Adenylyl cyclase activities were determined in membranes from CHW cells expressing the indicated receptors. Cells were exposed to vehicle (control) or 10 \( \mu \)M epinephrine (desensitized) for 20 min as described in Methods. Data are normalized to the control maximal response after subtraction of basal levels. Shown are results from four experiments. For the \( \beta_1 \)AR, the desensitization was greater for the Arg389 compared to the Gly389 variant (34\( \pm \)4.1\% vs 21\( \pm \)6.7\%, \( p<0.01 \)). For the \( \beta_2 \)AR, the Ile164 receptor displayed greater desensitization than the Thr164 allelic counterpart (37\( \pm \)4.0\% vs 26\( \pm \)4.0\%, \( p<0.05 \)).

Figure 2. Effects of desensitization on absolute activities of adenylyl cyclase stimulation by polymorphic \( \beta_1 \)AR and \( \beta_2 \)AR. Shown are data from experiments described in figure 1 plotted as absolute adenylyl cyclase activities (pmol/min/mg). Subscripts indicate control (C) or desensitization (D) conditions. For both \( \beta_1 \)AR and \( \beta_2 \)AR there was a relationship between genotype, desensitization status, and activities (\( p<0.005 \) by ANOVA). Basal and maximal stimulated activities were all different from their allelic or desensitization counterparts except for \( \beta_1 \)AR-Gly\( _C \) and Arg\( _D \) maximal stimulations, which were the same.

Figure 3. Efficacy of inverse agonists acting at polymorphic \( \beta_1 \)AR and \( \beta_2 \)AR. Accumulation of whole cell cAMP was determined over a 45 min period after addition of 100 \( \mu \)M IBMX and vehicle or varying concentrations of the inverse agonist CGP-20712 (\( \beta_1 \)AR) or ICI-118551
(β₂AR). Shown are the maximal responses from 4-5 experiments. The absolute levels of cAMP accumulation in the presence of inverse agonist were not different between genotypic variants. Expressions were β₁AR Arg389=4.8±0.6, β₁AR Gly389=3.7±0.7, β₂AR Thr164=2.1±0.34, β₂AR Ile164 2.2±0.39 pmol/mg.

Figure 4. Matrix representation of relative adenylyl cyclase activities of β₁AR stratified by genotype, desensitization status, and agonist stimulation. Results are from the computer fit minimum and maximal values from the mean curves from experiments in figure 2, normalized to agonist-stimulated adenylyl cyclase activities of β₁AR-Arg389 in the non-desensitized (control) state.

Figure 5. General model of the potential interaction of coupling polymorphisms and desensitization. Shown are two polymorphic receptors (R₁, Rᵣ), under conditions of agonist (A) or inverse agonist (IA) occupancy. The desensitized state refers to recent prior exposure to agonist in vivo. Different conformations of the receptor are depicted as *1, *2, *3, *4, which depend on the effects of the polymorphism and desensitization status. The italicized subscripts outside the brackets indicate the relative abundance of a given species. For example, at steady state, it would be expected that k>j.
Table 1. Adenylyl cyclase activation under control and desensitized conditions for the polymorphic $\beta_1$- and $\beta_2$-adrenergic receptors. Results are from 5-7 independent experiments.

There was a significant relationship between genotype and basal and maximal isoproterenol stimulated adenylyl cyclase activities ($p<0.001$ by ANOVA). The subscripts C and D denote activities under control (no agonist pretreatment) and desensitized (with agonist pretreatment) conditions, respectively. Iso=maximal isoproterenol stimulated values.

| Parameters | $\beta_1$-Gly389 | $\beta_1$-Arg389 | $\beta_2$-Thr164 | $\beta_2$-Ile164 |
|------------|-----------------|-----------------|-----------------|-----------------|
| Expression, fmol/mg (%) cell surface | 206 ± 16 (80 ± 2.1) | 170 ± 22 (88 ± 3.5) | 783 ± 88 (85 ± 1.7) | 1104 ± 11 (83 ± 2.1) |
| Adenylyl cyclase, pmol/min/mg | | | | |
| BasalC | 3.7 ± 0.61 | 8.2 ± 1.1 | 4.4 ± 0.89 | 2.0 ± 0.25 |
| BasalD | 2.5 ± 0.43 | 5.5 ± 0.74 | 3.8 ± 0.71 | 1.9 ± 0.54 |
| IsoC | 15.1 ± 2.2 | 21.8 ± 2.8 | 17.1 ± 0.45 | 8.9 ± 0.45 |
| IsoD | 11.9 ± 2.3 | 14.9 ± 2.2 | 13.1 ± 0.56 | 6.4 ± 1.29 |
| $pK_{act}$ | | | | |
| $pK_{actC}$ | 2.28 ± 0.17 | 2.34 ± 0.19 | 1.38 ± 0.22 | 1.48 ± 0.09 |
| $pK_{actD}$ | 2.32 ± 0.23 | 2.50 ± 0.19 | 1.08 ± 0.14 | 1.28 ± 0.20 |
Acknowledgements

The authors thank Cheryl Theiss for cell culture and Esther Getz for manuscript preparation.
Figure 1
Figure 2

**A**

$\beta_1\text{AR}$

Adenylyl Cyclase Activity

pmol/min/mg

log [isoproterenol]

- $\text{Arg}_C$
- $\text{Gly}_C / \text{Arg}_D$
- $\text{Gly}_D$

**B**

$\beta_2\text{AR}$

Adenylyl Cyclase Activity

pmol/min/mg

log [isoproterenol]

- $\text{Thr}_C$
- $\text{Thr}_D$
- $\text{Ile}_C$
- $\text{Ile}_D$
Figure 3
| Condition                      | Polymorphic Receptor 1 | Polymorphic Receptor 2 |
|-------------------------------|------------------------|------------------------|
| Inverse agonist binding       | $[\text{IAR}_1]_i$ $\rightarrow$ $[\text{R}_1^*1]_j$ | $[\text{IAR}_1]_{ii} \rightarrow [\text{R}_{II}^*3]_{jj}$ |
| Not desensitized; agonist binding | $[\text{R}_1^*1]_j \rightarrow [\text{AR}_1^*1]_k$ | $[\text{R}_{II}^*3]_{jj} \rightarrow [\text{AR}_{II}^*3]_{kk}$ |
| Desensitized; agonist binding | $[\text{R}_1^*2]_l \rightarrow [\text{AR}_1^*2]_m$ | $[\text{R}_{II}^*4]_{ll} \rightarrow [\text{AR}_{II}^*4]_{mm}$ |

Figure 5
Hierarchy of polymorphic variation and desensitization permutations relative to beta-1 and beta-2 adrenergic signaling
Deborah A. Rathz, Kimberly N. Gregory, Ying Fang, Kari M. Brown and Stephen B. Liggett

*J. Biol. Chem.* published online January 13, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M206054200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts