Dimers of Class A G Protein-coupled Receptors Function via Agonist-mediated Trans-activation of Associated G Proteins

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The histamine H1 receptor and the \( \alpha_{2b} \)-adrenoreceptor are G protein-coupled receptors that elevate intracellular [Ca\(^{2+}\)] via activation of \( \gamma_{1b}/\alpha_{1b} \). Assessed by co-immunoprecipitation and time-resolved fluorescence resonance energy transfer they both exist as homodimers. The addition of the G protein \( \gamma_{1b}/\alpha_{1b} \) to the C terminus of these receptors did not prevent dimerization. Agonists produced a large stimulation of guanosine 5’-3-O-\[^{35}S\]thio)triphosphate (\[^{35}S\]GTP\(\gamma\)S) binding to receptor-G protein fusions containing wild type forms of both polypeptides. For both receptors this was abolished by incorporation of \( \gamma_{2b}/\alpha_{1b} \) into the fusions. Mutation of a highly conserved leucine in intracellular loop 2 of each receptor also eliminated agonist function but did indicate a role for transmembrane helix 1 in dimerization. These data demonstrate that dimers of these class A receptors function via trans-activation of associated G proteins.

This concept that G protein-coupled receptors (GPCRs)\(^*\) exist as dimers or higher order oligomers has moved rapidly from hypothesis to being widely accepted (1–4). A range of approaches has contributed to this understanding. This includes the ability to co-immunoprecipitate differentially epitope-tagged forms of a GPCR from cells in which they are co-expressed, and, in intact cells, the application of a number of resonance energy transfer-based techniques. However, the role of dimerization in function and the mechanisms then responsible for initiation of signal transduction by the dimer have been more recalcitrant to analysis. Significant progress in this area has recently been achieved for the class C GPCRs. These contain both a long extracellular N-terminal domain, to which agonist ligands bind, and the prototypic seven transmembrane (TM) helix bundle architecture that is the common feature of all GPCR families (for review, see Ref. 5). The functional \( \gamma\)-aminobutyric acid, type b receptor is a hetero-dimer of two distinct gene products in which trafficking to the plasma membrane requires interaction between the partner polypeptides (6–9). This indicates that a key role of dimerization is achieving appropriate cellular localization. This is also true for the class A rhodopsin-like GPCRs because non-functional, truncated splice variants can restrict plasma membrane delivery of full-length GPCRs and, thus, limit their function (10–12). Chimeric class C GPCRs consisting of the extracellular domain of one GPCR and the TM and intracellular elements of a second, closely related GPCR or in which the intracellular loops of dimer partners are exchanged have provided strong evidence that the mechanism of action of the dimer involves trans-activation (13–14); that is, ligand binding to one element of the dimer results in activation of G protein produced by the other GPCR within the dimer. Again, the \( \gamma\)-aminobutyric acid, type b receptor has been particularly informative in this regard as only one of the two gene products that forms the dimer is able to bind the agonist \( \gamma\)-aminobutyric acid. Equivalent systems are not available for the majority of class A GPCRs. However, for the luteinizing hormone receptor, which also has a long N-terminal domain that binds the ligand, partial reconstitution of function has been achieved by co-expression of distinct pairs of mutants (15–16). Furthermore, in recent studies co-expression of a mutant receptor defective in hormone binding and another mutant defective in signal generation rescued hormone-activated cAMP production (17).

Fusion proteins in which G protein \( \alpha \) subunits are linked in-frame to the C terminus of a GPCR have become widely used tools to study the details of information transfer between these proteins (18–19). Because these can be considered as bi-functional proteins containing the sequence and function of both GPCR and G protein they can be utilized to generate contrasting pairs of non-functional mutants. Herein we generate and analyze fusion proteins incorporating either wild type GPCRs or mutants unable to activate G proteins and wild type or mutant G proteins unable to be activated by GPCRs. We show that co-expression of pairs of these non-functional mutant fusion proteins is complementary and results in the generation of functional dimers. This reconstitution can be both easily quantitated in membrane preparations and monitored in single intact cells. Studies of hetero-dimeric GPCR pairs support a mechanism in which agonists at amine-ergic class A GPCRs mediate trans-activation of the GPCR-associated G proteins.

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\[^{‡}\] The abbreviations used are: GPCR, G protein-coupled receptor; TM, transmembrane; tr-FRET, time-resolved fluorescence resonance energy transfer; GTP\(\gamma\)S, guanosine 5’-3-O-\(^{35}\)S(thio)triphosphate; GFP, green fluorescent protein.

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were resuspended in solubilization buffer (100 mM Tris, 200 mM NaCl, 100 mM EDTA, 1.25% Nonidet P-40) plus 0.2% sodium dodecyl sulfate. Samples were pre-cleared with Pansorbin (Calbiochem) followed by immunoprecipitation with CQ antisera (25). Finally, the immunocomplexes were washed twice with solubilization buffer, and bound 

$[^{35}]$GTP-S was measured by liquid scintillation spectrometry.

$[^{3}H]$Ligand Binding Studies—$[^{3}H]$Prazosin binding studies, monitoring expression of the $\alpha_1$-adrenergoreceptor-containing constructs, were performed as in Carillo et al. (26). $[^{3}H]$Prazosin binding assays, monitoring expression of the histamine H1 receptor-containing constructs, were initiated by the addition of $8 \mu$g of cell membranes to an assay buffer (50 mM Tris-HCl, 100 mM NaCl, 5 mM MgCl$_2$ (pH 7.4)) containing $[^{3}H]$Prazosin (0.1–10 nM). Nonspecific binding was determined in the presence of 100 $\mu$M trilopride. Reactions were incubated for 30 min at 25 °C, and bound ligand was separated from free ligand by vacuum filtration through GF/B filters. The filters were washed twice with assay buffer, and bound ligand was estimated by liquid scintillation spectrometry.

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individually and then combined before assay, no agonist-stimulated binding of [35S]GTPyS was observed (Fig. 1D). Such results are consistent with the hypothesis that GPCR dimerization is required for agonist function. Furthermore, within the dimer, one GPCR element must activate the G protein physically linked to the partner GPCR. To extend this concept

![Table 1](https://www.jbc.org/)

The binding affinity of agonist and antagonists ligands to α1b-adrenoreceptor- and histamine H1 receptor-G11 fusion proteins is unaffected by the addition of N-terminal epitope tags or the introduction of specific mutations. Data are presented as means ± S.E. from a minimum of three separate experiments.

| Fusion construct | $K_d$ [3H]prazosin (nM) | $B_{max}$ (pmol/mg) | $K_i$ phenylephrine (μM) |
|------------------|-------------------------|---------------------|--------------------------|
| α1b-G11          | 0.19 ± 0.02             | 7.8 ± 0.4           | 9.6 ± 0.3                |
| L151D/α1b-G11    | 0.23 ± 0.03             | 10.7 ± 1.5          | 11.4 ± 1.0               |
| α22-G208AG11     | 0.18 ± 0.03             | 5.9 ± 0.3           | 9.0 ± 0.6                |
| c-Myc-α1b-G11    | 0.14 ± 0.01             | 4.6 ± 0.2           | 7.8 ± 0.4                |
| FLAG-α1b-G11     | 0.10 ± 0.01             | 2.0 ± 0.1           | 6.0 ± 0.2                |

| Fusion construct | $K_d$ [3H)mepyramine (nM) | $B_{max}$ (pmol/mg) | $K_i$ histamine (μM) |
|------------------|---------------------------|---------------------|----------------------|
| H1-G11           | 1.5 ± 0.2                 | 4.8 ± 0.6           | 32.6 ± 3.6            |
| L133D H1-G11     | 1.7 ± 0.4                 | 5.4 ± 0.4           | 46.9 ± 3.8            |
| H1-G208AG11      | 1.5 ± 0.1                 | 3.6 ± 0.6           | 44.0 ± 2.5            |
| c-Myc-H1-G11     | 1.4 ± 0.1                 | 8.5 ± 0.1           | 31.3 ± 3.2            |
| FLAG-H1-G11      | 1.8 ± 0.02                | 8.6 ± 0.1           | 43.0 ± 7.1            |
an equivalent set of experiments was performed using fusions between the histamine H1 receptor and G11α. The basic results were the same. The fusion containing wild type forms of both the GPCR and G protein produced a large stimulation of [35S]GTPγS binding in the presence of the agonist histamine (Fig. 2). This was absent upon separate expression of either histamine H1 receptor-G208AG11 fusion protein or a fusion between L133D histamine H1 receptor and wild type G11α. Co-expression of these two mutants reconstituted agonist activation of the G protein (Fig. 2). Again, after co-expression of the two mutants, membranes expressing a 2-fold higher number of [3H]-labeled antagonist binding sites produced nearly as high a level of [35S]GTPγS binding in the presence of the agonist. The monomeric form of the isolated histamine H1 receptor (Fig. 4B) was then monitored in these cells. The warmer color represents elevated [Ca2+]i. The response of GFP-positive cells to 10μM phenylephrine (Phe) was then measured over time. n = the number of individual cells quantitated.

As an extension to these studies we attempted to monitor dimerization and functional reconstitution in a single cell. To do so we employed Ca2+ imaging using EF88 cells. These are a line of mouse embryo fibroblasts that derive from a G11α; L133D histamine H1 receptor-G11α; 3, histamine H1 receptor-G208AG11; 4, and 5, histamine H1 receptor-G208AG11; L133D histamine H1 receptor-G11α.

We also wished to demonstrate directly the ability of both the isolated GPCRs and the GPCR-G protein fusions to form dimers. Constructs were N-terminally epitope-tagged with either the c-Myc or FLAG tags. After co-expression in HEK293 cells of both tagged forms of the α1b-adrenoreceptor, but not their separate expression, followed by cell mixing, immunoprecipitation with anti-FLAG antibodies resulted in the presence of anti-c-Myc immunoreactivity in the precipitate (Fig. 4A). SDS-PAGE demonstrated the presence of bands identified by the c-Myc antibody of apparent size 53 and 110 kDa, consistent with monomeric and dimeric forms of the α1b-adrenoreceptor. Anti-c-Myc immunoreactivity was also observed near the top of the gel, and this may represent either a higher order oligomer or aggregated protein (Fig. 4A). When equivalent experiments were performed with the α1a-adrenoreceptor-G11α fusion protein similar results were obtained except that the anti-c-Myc-reactive bands were now of apparent mass 95 and 190kDa, consistent with the anticipated size of monomeric and dimeric forms of this fusion protein (Fig. 4A). Similar results were obtained for FLAG- and c-Myc-tagged forms of the histamine H1 receptor (Fig. 4B). The monomer form of the isolated
To examine the possibility of hetero-dimerization between the histamine H1 receptor and the α1b-adrenoceptor and the mechanism of G protein activation by GPCR dimers we co-expressed a FLAG-tagged form of the histamine H1 receptor and the c-Myc-tagged form of the α1b-adrenoceptor. After immunoprecipitation with anti-FLAG antibodies and SDS-PAGE, c-Myc immunoreactivity was detected in polypeptides of apparent molecular mass 50 and 100 kDa, consistent with the immunoprecipitation of histamine H1 receptor-α1b-adrenoceptor hetero-dimers that are only partially separated by the electrophoresis conditions employed (Fig. 6A). tr-FRET studies after co-expression of the FLAG-tagged form of the histamine H1 receptor and the c-Myc-tagged form of the α1b-adrenoceptor confirmed the presence of histamine H1 receptor/α1b-adrenoceptor hetero-dimers at the cell surface (Fig. 6B), although the absolute level of the signal indicated that these heterodimers formed less efficiently than the corresponding homodimer pairs (see the y axis of Figs. 5, A and B, compared with Fig. 6B). As in the homo-dimer studies, no tr-FRET signal was observed when separate cell populations expressing each of these receptors were mixed before analysis (Fig. 6B).

When L133D histamine H1 receptor-G11α was co-expressed in EF88 cells with α1b-adrenoceptor-G208AG11α, phenylephrine was able to elevate intracellular [Ca2+]i, but histamine was not (Fig. 7A). This can only occur if the α1b-adrenoceptor activates the G protein physically linked to the L133D histamine H1 receptor. When the protocol was reversed by co-expression of L151D α1b-adrenoceptor-G11α and histamine H1 receptor-G208AG11α, histamine now caused elevation of intracellular [Ca2+]i, but histamine was not (Fig. 7B). To extend this type of analysis the histamine H1 receptor-G11α fusion was co-expressed with the isolated L151D α1b-adrenoceptor that is unable to activate G protein and, thus, stimulate binding of [35S]GTPγS. Histamine stimulation of [35S]GTPγS binding was significantly reduced in comparison to membranes expressing the same level of only the histamine H1 receptor-G11α fusion (Fig. 8A). Such data are consistent with the L151D α1b-adrenoceptor generating inactive hetero-dimers with histamine H1 receptor-G11α fusion protein (Fig. 8B).
EF88 cells. Histamine stimulation of intracellular Ca\(^{2+}\) in the co-transfection reflects that some functional histamine in the hetero-dimer does not activate the G protein physically.

\[ \text{H1 receptor-G11} \]

BSR reduced markedly (Fig. 8A). The number of histamine H1 receptor binding sites decreased as levels of the L133D histamine H1 receptor homo-dimer is still formed in the presence of inactive histamine H1 receptor- and L133D histamine H1 receptor-G11α homo-dimers. To enhance the levels of appropriately membrane-targeted G protein we generated a construct in which G11α was linked to the C terminus of a c-Myc-tagged form of the N-terminal and first TM region of the α1b-adrenoreceptor (c-Myc-Nt-TM1α1b-G11α). This was transfected into HEK293 cells. Immunoblots of membrane fractions clearly demonstrated its expression as a doublet of 53 and 47 kDa whether detection was via anti-c-Myc (Fig. 9A) or anti-G protein antisera (data not shown). Based on immunodetection by the anti-c-Myc antibody, levels of c-Myc-Nt-TM1α1b-G11α were significantly greater than those of the c-Myc-α1b-adrenoreceptor-G11α fusion protein (Fig. 9A). 

[\[^{[35]S}\]GTP\(\gamma\)S binding assays, at the end of which the c-Myc-Nt-TM1α1b-G11α construct was immunoprecipitated with anti-c-Myc antibodies, confirmed this construct did not bind \[^{[35]S}\]GTP\(\gamma\)S in response to phenylephrine (Fig. 9B). Parallel experiments showed that the anti-c-Myc antibodies did capture phenylephrine-stimulated binding of \[^{[35]S}\]GTP\(\gamma\)S to the full-length c-Myc-tagged α1b-adrenoreceptor-G11α fusion protein (Fig. 9B). However, co-expression of c-Myc-Nt-TM1α1b-G11α with the isolated α1b-adrenoreceptor equally did not result in significant stimulation of \[^{[35]S}\]GTP\(\gamma\)S binding in anti-c-Myc immunoprecipitates (Fig. 9B), and this was also true when c-Myc-Nt-TM1α1b-G11α was co-expressed with the α1b-adrenoreceptor-G208AG11α fusion protein (Fig. 9B). Thus, simply increasing the concentration of membrane-associated G protein did not allow the α1b-adrenoreceptor or α1b-adrenoreceptor fusion protein homo-dimers to activate this G protein. It was possible that co-expression of c-Myc-Nt-TM1α1b-G11α with forms of the α1b-adrenoreceptor did not result in their physical proximity and that this might account for the lack of activation of the membrane-tethered G11α by phenylephrine. To assess this we co-expressed c-Myc-Nt-TM1α1b-G11α with the FLAG-tagged α1b-adrenoreceptor-G11α fusion protein. This pairing produced a strong tr-FRET signal after the addition of a combination of Eu\(^{3+}\)-labeled anti-c-Myc and allophycocyanin-labeled anti-FLAG antibodies, and tr-FRET was measured.

H1 receptor-G11α and indicate that the histamine H1 receptor in the hetero-dimer does not activate the G protein physically associated with it. The remaining signal produced by histamine in the co-transfection reflects that some functional histamine H1 receptor-G11α homo-dimer is still formed in the presence of L151D α1b-adrenoreceptor. Indeed, when we co-expressed histamine H1 receptor-G11α with increasing amounts of L151D α1b-adrenoreceptor cDNA, the ability of histamine to cause \[^{[35]S}\]GTP\(\gamma\)S binding in membranes expressing the same number of histamine H1 receptor binding sites decreased as levels of L151D α1b-adrenoreceptor cDNA were increased (Fig. 8A). Similar results were obtained after co-transfection of L151D α1b-adrenoreceptor with the histamine H1 receptor-G11α in EF88 cells. Histamine stimulation of intracellular Ca\(^{2+}\) was reduced markedly (Fig. 8B).

Co-expression of two distinct GPCRs must result in the presence of the respective homo-dimers as well as providing the potential for hetero-dimer formation. We wished to ensure that the reconstitution of Ca\(^{2+}\) signaling observed upon co-expression of L133D histamine H1 receptor-G11α with α1b-adrenoreceptor-G208AG11α did not reflect that only α1b-adrenoreceptor and histamine H1 receptor homo-dimers were present and that the α1b-adrenoreceptor-G208AG11α homo-dimers were simply able to contact and activate G11α linked to L133D histamine H1 receptor-G11α homo-dimers. To enhance the levels of α1b-adrenoreceptor indicated that a peptide corresponding to TMVI of the α1b-adrenoreceptor-G11α homo-dimers was still formed in the presence of L133D histamine H1 receptor-G11α homo-dimers.

**DISCUSSION**

That GPCRs can exist as dimers is now widely accepted (1–4). However, the basis and importance of this for function has been less easy to establish. Early studies on the β2-adrenoreceptor indicated that a peptide corresponding to TMVI of the receptor was able to prevent dimerization and limit agonist stimulation of adenylyl cyclase activity (33). The general applicability of these observations, however, remains to be exemplified. Indeed, although recent studies on the leukotriene B\(_4\) BLT1 receptor also support a key role for TMVI in homo-dimer formation (34), studies on the dopamine D2 receptor suggest a key role for TMIV (35). Furthermore, application of atomic force microscopy to the study of rhodopsin in native disc membranes (36–37) suggests that inter-dimeric contacts may involve TM segments IV and V. Distinct approaches provide evidence of roles for TM1 and TM2 in the stabilization of dimers of the yeast ste2 receptor (38) and of TM segments I, II, ...
and/or IV in the complement C5a receptor (39). This range of results is fascinating, and combinations of direct experimentation and bio-informatic approaches (40–41) are likely to be required to provide understanding. Given the variation in potential orientation and organization between GPCRs, it is thus interesting that in experiments described herein, we have also provided evidence consistent with a role for TM1 in the dimerization of this receptor (Fig. 9C). As well as homo-dimers, many pairs of GPCRs are able to form hetero-dimers (1–4), and there is evidence that this results in production of distinct pharmacology and alterations in function (42–47). Although yet to be tested directly, there are suggestions that in such heterodimers, the individual GPCRs may contribute different elements to the dimer interface (48). We have demonstrated the capability of the $\alpha_{1b}$-adrenoreceptor and the histamine H1 receptor to form hetero-dimers. It is, thus, of importance to note that both of these GPCRs are expressed in arterial smooth muscle cells (49–50) and may, thus, form hetero-dimers in vivo.

Although class C is the smallest family of GPCRs, studies on the importance of dimerization and the mechanisms of signal transduction within the dimer have advanced most rapidly in this group (5). This reflects both that the GABA type b receptor is an obligate hetero-dimer between two distinct but related GPCR gene products and that all members of this family have a large N-terminal extracellular domain that is responsible for binding of agonist ligands. It has, thus, been relatively easy to generate chimeric class C receptors that mix and match the extracellular and transmembrane and intracellular domains of individual GPCRs. The rhodopsin-like class A GPCRs comprise greater than 80% of the entire family present in the human genome (51–52). However, few class A GPCRs have structural features to facilitate such an approach, and GPCRs with amineergic agonists bind the ligands deep within the cleft produced by the architecture of the seven TM helices. By employing fusion proteins between both the $\alpha_{1b}$-adrenoreceptor and the histamine H1 receptor with the G protein $G_{11\alpha}$, we now show that these GPCRs dimerize and that this is not compromised by the addition of the G protein to the C-terminal tail of the GPCR. Equally, by employing tr-FRET to detect GPCR dimers in intact cells we were able to demonstrate the presence of these complexes at the cell surface. This also was not compromised by the addition of the G protein sequence to the C-terminal tail of the GPCRs. Furthermore, by introducing mutations that prevent agonist activation of the G protein into either the GPCR or the G protein, we produced pairs of distinct, non-functional fusion proteins that were able to restore ago-
immunoblotted with anti-c-Myc antibodies. Amplified signal and, thus, not ideal for quantitation of the aC a2 both a suitable GPCR and G protein into these cells to generate after co-expression of two mutants that were each non-functional pairs of non-functional mutants. account for the reconstitution of function in cells expressing c-Myc-Nt-TM1 of the corresponding wild type GPCRs. Functional reconstitution was monitored in two ways. First, agonists were able to produce elevation of intracellular [Ca2+].

Binding of both agonist and antagonist-mediated function when co-expressed. Many of the studies utilized combinations of epitope tagging and/or mutagenesis of the fusion proteins. As such, it was important to confirm that these modifications did not cause substantial alterations in the binding of ligands compared with the parental fusion proteins. We demonstrated that the binding of both agonist and antagonists ligands was not markedly different for any of the modified constructs (Table 1), and these values were similar to those of the corresponding wild type GPCRs. Functional reconstitution was monitored in two ways. First, agonists were able to produce elevation of intracellular [Ca2+] in EF88 cells only after co-expression of two mutants that were each non-functional in isolation. EF88 cells lack expression of phospholipase C-coupled G proteins, and thus, it is necessary to introduce both a suitable GPCR and G protein into these cells to generate a Ca2+ signal (31). This assay had the obvious benefit that Ca2+ imaging allowed us to monitor functional dimerization in single cells and in the absence of excess G protein. However, although [Ca2+], can be calculated from such studies, this is an amplified signal and, thus, not ideal for quantitation of the effectiveness of dimerization. One of the earliest steps that can be measured in the signal transduction cascade is agonist-induced guanine nucleotide exchange on the G protein. This can be monitored conveniently by the binding of [35S]GTPyS. Historically, the characteristics of guanine nucleotide exchange by different G protein families had meant that this assay was only suited for Gs and Gq family G proteins (53). However, recent addition of end of assay immuno-capture steps has allowed it to be adapted to also measure activation of the Gi and Gq family G proteins (53). In all the [35S]GTPyS binding assays we initially measured the level of expression of each of the GPCR-G protein fusions by using saturation [3H]-labeled antagonist binding studies. This allowed us to add membrane samples containing defined quantities of the constructs to the assays. We have previously demonstrated that there is a linear increase in agonist-stimulated [35S]GTPyS binding with the addition of increasing amounts of a GPCR-Gi1α fusion protein (31). When co-expressing the histamine H1 receptor-G208AG11α and L133D histamine H1 receptor-Gi1α fusion proteins, it required the presence of twice the number of [3H]-labeled antagonist binding sites to generate approximately the same amount of agonist-stimulated [35S]GTPyS binding as when only the wild type histamine H1 receptor-Gi1α fusion protein was expressed. This provided evidence that the functional element is a dimer. If the functional histamine H1 receptor is a dimer, then stochastically, when co-expressing the two non-functional mutant fusions, half of the dimers produced should be non-functional because they will represent homo-interactions between either of the non-functional forms, i.e. histamine H1 receptor-G208AG11α or L133D histamine H1 receptor-Gi1α. Only 50% of the dimers would be expected to be hetero-dimers containing one copy of histamine H1 receptor-G208AG11α and one of L133D histamine H1 receptor-Gi1α and, thus, be functional. These studies are also consistent with the idea that amine-ergic class A GPCRs function via trans-activation of their associated G proteins. The copy of the G protein in the dimer that can be activated is linked to the non-functional form of the GPCR, whereas the functional form of the GPCR is associated with non-functional G protein. Further studies, however, were required to provide greater support for this mechanism. To do so we took advantage of the known capacity of structurally related GPCRs to form hetero-dimers. Initial studies demonstrated that when co-expressed the histamine H1 receptor and the α1b-adrenoceptor could be co-immunoprecipitated. Furthermore, co-expression in EF88 cells of L133D histamine H1 receptor-Gi1α and α1b-adrenoceptor-G208AG11α resulted in phenylephrine but not histamine-mediated elevation of [Ca2+]. This can only occur if the α1b-adrenoceptor activates the G protein physically linked to the inactive histamine H1 receptor (Fig. 10). When the experiment was reversed such that the inactive α1b-adrenoceptor was linked to the wild type G protein and the wild type histamine H1 receptor was linked to the mutant G protein, histamine was functional, but phenylephrine was not. We extended this idea by examining the effectiveness of histamine to stimulate binding of [35S]GTPyS when the histamine H1 receptor fusion protein was co-expressed with increasing amounts of the isolated but inactive L151D α1b-adrenoceptor. The effect of histamine was reduced. Such information is consistent with the concept that increasing levels of a histamine H1 receptor-Gi1α-L151D α1b-adrenoceptor hetero-dimer reduces the amounts of the histamine H1 receptor-Gi1α homo-dimer and that histamine binding to the hetero-dimer is unable to activate the G protein that is physically associated with the histamine H1 receptor. In this situation phenylephrine was inactive because L151D α1b-adre-
Trans-activation of GPCRs

noreceptor is unable to stimulate any G protein. A number of reports have indicated that GPCR-G protein fusions can interact with and activate endogenously expressed G proteins as well as the G protein element of the fusion (54–55). However, in these studies the GPCR-G protein fusions were expressed at very high levels that are within the range in which nonspecific “bystander” (56) effects have been reported due to physical proximity and crowding in the membrane. Use of EF88 cells eliminated the possibility of interaction with endogenous G proteins as they do not express Gα1 or Gα11, and thus, effects have to reflect activation of the fused G proteins. Moreover, after introduction of the G208A mutation into the G protein element of the fusions, agonist stimulation of [35S]GTPγS binding in membranes of transfected HEK293 cells was virtually abolished. This indicates that at the level of expression achieved, there was virtually no activation of endogenous Gα1 or Gα11α in HEK293 cells even though both are expressed (25).

In the hetero-dimerization experiments in HEK293 cells, excess G protein is introduced in a 1:1 molar ratio with the second GPCR. These studies provided further evidence that the reconstitution of signal with co-expression of non-functional pairs of GPCR-G protein fusions must reflect an internal trans-activation within the reconstituted dimer and are also consistent with a key role of TM1 as a dimer interface in the α11b-adrenoceptor.

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FIG. 10. Only hetero-dimers of GPCR-G protein fusion proteins should reconstitute function. Co-expression of a wild type (white) α11b-adrenoreceptor linked to a mutant (gray) G208AG11α and a mutant (gray) L133D histamine H1 receptor linked to wild type (white) G11α will result in the presence of each of α11b-adrenoreceptor and histamine H1 receptor homo-dimers and α11b-adrenoreceptor-histamine H1 receptor hetero-dimers. Only the hetero-dimers will be functional. In the example shown agonist at the α11b-adrenoreceptor (Phe) should generate a signal, but agonist at the histamine H1 receptor (Hist) will not. Demonstration of this principal is shown in Fig. 7A. The reverse combination of constructs results in the histamine H1 receptor element of the hetero-dimer being functional but not the α11b-adrenoreceptor element. This is demonstrated in Fig. 7B. In the diagram dimer formation is indicated to result from a linear packing arrangement. Other possible models have been described and discussed (40). PLC, phospholipase C; IP3, inositol 1,4,5-trisphosphate.

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