Pretty Subunits All in a Row: Using Concatenated Subunit Constructs to Force the Expression of Receptors with Defined Subunit Stoichiometry and Spatial Arrangement

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ABSTRACT

The members of the Cys-loop ligand-gated ion channel (LGIC) gene family play a major role in fast synaptic transmission, and these receptors represent an important class of targets for therapeutic agents. Each member of this gene family is a pentameric complex containing one or more different subunits, and a large number of subunits for each member have been identified. This large number of subunits could give rise to a bewildering array of possible subunit compositions and spatial arrangements within a single complex, not all of which may occur in vivo. Heterologous expression systems have been used to create specific combinations of individual subunits to mimic naturally occurring receptors. However, this approach is not without its problems. In this issue of Molecular Pharmacology, Groot-Kormelink et al. (page 559) describe a method for constructing “concatameric” receptors, in which five individual subunits are arranged in a predetermined order connected by a flexible linker. Expression of this construct results in the formation of receptors with a unique, predefined subunit stoichiometry and subunit arrangement within the receptor complex. Receptors formed from this construct are fully functional and have properties essentially identical to those formed from individual subunits. The application of this very general approach to other members of the LGIC family should markedly enhance our ability to understand how subunit composition influences receptor function, as well as provide a means for the expression of receptors of predefined subunit composition and arrangement as tools for the development of novel selective pharmacological and therapeutic agents.

ABBREVIATIONS: LGIC, Cys-loop ligand gated ion channel; AChR, nicotinic acetylcholine receptor; GABA\(_{A}\)R, GABA\(_{A}\) receptor.
These tools could then be used not only for analysis of the properties of receptors themselves but also for the development of novel therapeutic agents targeted toward a specific receptor (sub)subtype. However, for this approach to be truly fruitful, one must first determine the subunit stoichiometry of a given target and then develop methods for the expression of receptors with this exact stoichiometry and subunit arrangement. Neither of these tasks is by any means easy or straightforward.

Consider the simplest nontrivial situation—a pentameric receptor that contains two different subunits, A and B. There are six possible subunit stoichiometries (A₅, A₄B, A₃B₂, A₂B₃, AB₅, and B₅). With identical handedness governed by current version of the ligand-gated ion channel database Various human genes/gene products for each receptor cloned to date and listed in the resulting in variable subunit stoichiometry (Hedblom and Kirkness, 1997; Zwart and Vijverberg, 1998; Nelson et al., 2003). In addition, it is clear that these systems can produce receptors that probably do not exist in vivo—either homomorphic receptors (Beato et al., 2002) or subunit-deficient complexes (Jackson et al., 1990; Charnet et al., 1992). Furthermore, the presence of endogenous subunits in the expression system may further complicate the analysis of the effects of altering subunit composition on receptor function (Buller and White, 1990). Therefore, these systems may not always produce the type of receptors expected, and conclusions obtained from such studies may not be as firm as one would like.

One method to obtain the expression of receptors of known/predefined subunit stoichiometry and even subunit orientation is the use of concatameric subunit constructs. This forces subunits to be in a particular stoichiometry and spatial arrangement by expressing a polypeptide containing more than one subunit sequences in the mature protein. This is essentially what is seen naturally in some voltage-gated channels. In the case of K⁺ channels, the channel is a true tetramer formed from four individual subunits (MacKinnon, 1991; Doyle et al., 1998), whereas in Na⁺ and Ca²⁺ channels, the “tetramer” is formed by four homologous repeat domains within a single large polypeptide chain encoding the main subunit (Catterall, 1995).

Originally applied to voltage-gated K⁺ channels (Liman et al., 1992), this approach has been used for several members of the LGIC family (Im et al., 1995; Baumann et al., 2001; Zhou et al., 2003; Grudzinska et al., 2005). In most cases, tandem dimeric subunits were created with a polylong chain linker containing 10 to 25 glutamines between the two subunits. At this length, polylong is water-soluble and assumes a random coil conformation (Altschuler et al., 1997), making it an ideal linker for this purpose. Expression of the dimeric construct with one or more individual subunits can then be used to determine not only the subunit stoichiometry properties (Cooper et al., 1991; Backus et al., 1993; Chang et al., 1996; Boorman et al., 2000; Burzomato et al., 2003), and, in one case, atomic force microscopy (Barrera et al., 2005). Studies such as these provide most of what we know about subunit stoichiometry of receptors containing various subunits.

However, although one assumes/hopes that the receptors expressed in these systems will be faithful surrogates for naturally occurring receptors, there is some evidence that in heterologous expression systems, the ratio of subunits in a given receptor complex depends on the ratios of exogenous subunit RNA or DNA introduced into the expression system, resulting in variable subunit stoichiometry (Hedblom and Kirkness, 1997; Zwart and Vijverberg, 1998; Nelson et al., 2003). hetrogeneous monomers resulting in variable subunit stoichiometry (Hedblom and Kirkness, 1997; Zwart and Vijverberg, 1998; Nelson et al., 2003).

### Table 1

Human LGIC subunit genes/gene products

<table>
<thead>
<tr>
<th>Various human genes/gene products for each receptor cloned to date and listed in the current version of the ligand-gated ion channel database <a href="http://www.sht.ch.ac.uk/compneur-srv/LGICdb/LGICdb.php">http://www.sht.ch.ac.uk/compneur-srv/LGICdb/LGICdb.php</a> (Le Novère and Changeux, 2001). Please note that not all of the gene products have been unequivocally demonstrated to be incorporated into LGICs in vivo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin type 3 receptor (GABAAR)</td>
</tr>
<tr>
<td>Glycine receptor</td>
</tr>
<tr>
<td>Muscle AChR</td>
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<tr>
<td>Neuronal AChR</td>
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**Fig. 1.** Possible subunit stoichiometries and spatial arrangement of a receptor containing five subunits, each of which can be either an “A” subunit or a “B” subunit. Note that even for this simple system, there are six possible stoichiometries and eight potential arrangements of the subunits in the pentamer.
but also the spatial arrangement of the subunits in the complex. For example, if expression of A→B tandem subunits (where the carboxyl terminus of the A subunit is joined to the amino terminus of the B subunit via the linker) alone does not produce functional receptors, then one can rule out dimers, tetramers, or hexamers as forming the entire receptor complex (which would not be surprising given the fact that numerous studies analyzing receptors from both tissues and heterologous systems have demonstrated that these receptors are pentamers). If coexpression of the A→B dimer with the B subunit formed functional receptors but coexpression of an A subunit with the dimer did not, then (assuming a pentameric complex) one would conclude that the stoichiometry of the receptor was A₂B₃ and the subunit arrangement in the complex was A→B→A→B→B (and then back to the initial ‘A’ in the sequence to complete the loop).

The above analysis assumes that both parts of the dimeric subunit are incorporated into the same receptor complex. However, two recent studies suggest that this may not always be the case, complicating the analysis of the results. Through the use of α₃→β₂ and β₂→α₃ nicotinic AChR receptor subunit dimer constructs, Zhou et al. (2003) showed that in some cases, the subunits in the dimer may be incorporated into two separate complexes, resulting in a dimer of pentameric receptors expressed alone in X. laevis oocytes. This result indicates that the subunits in the dimer are not necessarily incorporated as a single unit, leading to the possibility of multiple subunit arrangements (e.g., α₃→β₂→α₃→β₂) giving different results. It is possible that the alternate sequences do not result in the formation of functional receptors, this might provide an opening to a more detailed analysis of the contributions of particular regions of each subunit that contribute to the subunit-subunit interactions that hold the complex together. Second, do other subunit arrangements (e.g., β₄→β₁→α₃→α₃) not produce functional receptors? If the receptor has a unique stoichiometry and subunit arrangement, concatamers such as this should not work. If other constructs do work, it would demonstrate that there is not a unique arrangement of a given group of subunits and lays the groundwork for future studies of how subunit arrangement affects receptor function.

The construction of pentameric subunit concatamers now allows several types of studies that could not be carried out before. First, as demonstrated in this study, one can incorporate a mutation into just one subunit of the receptor to examine the role of individual subunits—even those that may be present in multiple copies in the complex. Although one might consider this a situation that would occur only in experiments designed to probe receptor structure-function relationships, this also might be observed in the case of polymorphisms in heterozygotes for a particular mutation. Application of this approach to the ligand-binding site regions (which are believed to be located at subunit-subunit interfaces [Karlin, 2004]) should allow the delineation of the role of residues on either half of a specific ligand-binding site in the receptor in the actions of agonists and antagonists. Second, and perhaps of much wider interest, one can now create receptors of predefined subunit stoichiometry and arrangement and use these not only to test which stoichiometries/arrangements produce functional receptors but also to create well defined targets for the development of novel experimental probes and therapeutic agents with receptor (sub)subtype selectivity. It is now time for the fun stuff to begin!

References
Backus K, Arigoni M, Drescher U, Scheuerer L, Malherbe P, Mohler H, and Benson


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