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PERSPECTIVE

New Determinants of Receptor-Effector Coupling: Trafficking and Compartmentation in Membrane Microdomains

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The multicomponent modular nature of G protein-coupled receptor (GPCR) systems provides cells with numerous potential combinations by which to transduce signals. A typical cell appears to express a dozen or so different GPCR genes (of which nearly a thousand exist in the human genome), several different combinations of G protein subunits and multiple isoforms of effector molecules that can be activated by each type of G protein. The differential expression of these various proteins allows modulation of signals at many levels, resulting in messages that are customized for a specific cell type. Much of the effort to understand signaling via these complicated cellular networks has focused on defining the linear progression of molecular interactions involved in a given pathway. This approach has yielded important information regarding the types of pathways (i.e., the class of G protein and cognate downstream effectors) that are characteristically activated by a given receptor and, thereby, the types of alterations in cell function that are elicited. The current dogma is that high-affinity protein-protein interactions determine the identity of the G protein with which a particular GPCR interacts and, in turn, dictates the biochemical pathways that are activated by that receptor. However, numerous observations in various cells and tissues have indicated that different receptors coupling to the same G protein in a single cell can elicit different biochemical or cellular responses (Hayes and Brunton, 1982; Buxton and Brunton, 1983; Harper et al., 1985; Graeser and Neubig, 1993; Xu et al., 1996; Steinberg and Brunton, 2001). The classical view that GPCR signal transduction is one-dimensional cannot readily account for these observations. Two additional dimensions must be incorporated into our conceptual models: 1) the compartmentation of receptors and effector molecules in subcellular compartments and microdomains of the plasma membrane, and 2) the movement, or translocation, of receptors between cellular compartments (trafficking).

Compartmentation and Caveolae

The concept of receptor translocation is certainly not a new one. The decades old observations that binding sites are lost and responses desensitize following agonist exposure were explained, at least in part, by internalization and sequestration of receptors (Clark et al., 1985; Kassis et al., 1986). A more contemporary idea is that cellular plasma membranes do not uniformly express GPCR effector molecules but do so in specific membrane microdomains (i.e., compartmentation) (Neubig, 1994; Anderson, 1998; Okamoto et al., 1998; Ostrom et al., 2000). Incorporation of these two concepts with classical views of GPCR signaling is opening the door to a new understanding of the biology of signal transduction. We are just now gaining the knowledge and expertise to study the morphology and structural mechanisms by which cells compartment signaling components to begin to assess how the localization and translocation of a receptor and its effectors can influence its signaling.

Recent investigations have identified caveolae as key microdomains of the plasma membrane that appear to concentrate (perhaps preassemble) certain components of signal transduction pathways and, in some cases, serve as sites of internalization. Caveolae differ biochemically from another specialized region of the plasma membrane implicated in receptor-trafficking, clathrin-coated pits. Caveolae are enriched in sphingolipid and cholesterol, making these lipid domains more buoyant than other portions of the cell and facilitating their isolation using sucrose density centrifugation. Clathrin-coated pits and caveolae serve as sites that transport different types of molecules and thus appear to represent similar but distinct pathways for internalization of GPCR. A growing list of signaling molecules has been found to reside in caveolae or to closely associate (i.e., immunoprecipitate) with caveolins (Shaul and Anderson, 1998; Ostrom

ABBREVIATIONS: GPCR, G protein-coupled receptors, B_1R, bradykinin B_1 receptor; B_2R, bradykinin B_2 receptor; $cPLA_2$, cytosolic phospholipase A_2; MAP kinase, mitogen-activated protein kinase; AC, adenylyl cyclase; PLC, phospholipase C; PIP_2, phosphatidylinositol; GFP, green fluorescent protein.

et al., 2000). Various GPCR and receptor tyrosine kinases have been localized in caveolae or caveolin-rich cellular fractions along with many of the molecules critical for transducing the signals initiated by these types of receptors: e.g., G proteins, adenylyl cyclase, protein kinase C, nitric oxide synthase and the components of the mitogen-activated protein (MAP) kinase cascade (extracellular signal-regulated protein kinase, MAP kinase kinase, Raf, and Ras) (Okamoto et al., 1998; Shaul and Anderson, 1998; Ostrom et al., 2000). Some GPCR are enriched or excluded from caveolae whereas certain GPCR reportedly translocate out of or into caveolae upon activation by an agonist. Therefore, caveolae appear to act as centers that concentrate certain signaling molecules while excluding others, making them key domains that mediate compartmentation within the plasma membrane.

Translocation without Sequestration

In this issue, a report by Sabourin et al. (2002) describes a novel variation on the theme of receptor trafficking via caveolae. These workers report studies in which the rabbit bradykinin B_1 receptor (B_1R) was fused with a fluorescent protein, and trafficking of the chimeric protein was then studied following agonist exposure. The authors observe that upon activation by an agonist, B_1R translocated to caveolin-rich membrane microdomains but did not subsequently appear in intracellular compartments. These results are consistent with a recent report by Lamb et al., who studied trafficking of the human B_1R and B_2R (Lamb et al., 2001). However, these latter investigators noted a 10-fold decrease in agonist affinity of their B_1R-GFP construct relative to the wild-type receptor, whereas Sabourin et al. (2002) detected no functional change when GFP was fused to the rabbit B_1R. Lamb et al. (2001) were also unable to assess subcellular localization of their B_1R-GFP construct due to limited expression levels. The trafficking of the B_1R contrasts with that of the B_2R: in studies by Sabourin et al. (2002) and in previous reports from other groups, B_2R-GFP chimeras translocated to caveolin-rich membranes upon activation but then rapidly internalized (de Weerd and Leeb-Lundberg, 1997; Haasemann et al., 1998; Lamb et al., 2001). Trafficking of the B_1R also contrasts with other examples of caveolae-related GPCR translocation, including cardiac β_2-adrenergic and A_1 adenosine receptors that move out of caveolin-rich membranes upon agonist exposure and, in the case of β_2-adrenergic receptors, internalize via clathrin-coated pits (Cao et al., 1998; Lasley et al., 2000; Rybin et al., 2000; Ostrom et al., 2001). Taken together, these findings make evident that many permutations of receptor localization (caveolar or noncaveolar), translocation (into/out of caveolae or no translocation), and internalization (positive or negative, caveolae or coated pits) are employed in GPCR biology. Why and how such permutations occur is not yet clear.

What could be the purpose of a GPCR translocating to a vesicular domain if it does not undergo endocytosis? Perhaps the answer can be found in considering what receptor translocation accomplishes. Classically, receptor movement to a specific domain has been considered a means to associate with specialized regions of the plasma membrane capable of mediating endocytosis of the receptor as part of the desensitization/resensitization mechanism. However, recent evidence demonstrates a second key role for such movement: localization of the receptor promotes coupling to a particular signaling pathway. For example, cardiac β_2-adrenergic receptors couple sequentially to the stimulation of cAMP production and then to the activation of MAP kinases—a switching that appears to require endocytosis of the receptor (Daaka et al., 1998). Therefore, some cells utilize GPCR translocation as a means for gaining signaling diversity. This is likely a conserved theme in biology.

Localization as a Determinant of Signaling

An interesting implication of the report by Sabourin et al. (2002) is that the signaling of B_1R mirrors its trafficking and contrasts with that of both the trafficking and signaling of the B_2R. Both kinin receptors couple to the $G_{q/11}$ family of G proteins and the activation of phospholipase C (PLC, via $G_{q/11}$) and cytosolic phospholipase A_2 ($cPLA_2$) (Farmer and Burch, 1992). The increases in intracellular Ca^{2+} concentrations stimulated by both receptors desensitize following exposure to agonist, but B_1R signaling through $cPLA_2$ continues unabated (Mathis et al., 1996; Zhou et al., 2000). As illustrated in a hypothetical schematic (Fig. 1), both receptors translocate from noncaveolar regions of the plasma membrane to caveolin-rich membranes upon agonist activation. This change in location likely facilitates linkage of activated receptor-G protein complex with effector enzyme (PLC) and substrate (phosphatidylinositol, PIP_2), both of which are enriched in caveolae (Pike and Casey, 1996; Pike and Miller, 1998). PLC-mediated production of the second messenger inositol trisphosphate leads to Ca^{2+} mobilization, whereas MAP kinase activation (via $G_{\beta\gamma}$ and diacylglycerol) contributes to the activation of $cPLA_2$ (Qiu et al., 1998). At this point the signaling of the two receptor subtypes appears to diverge. B_2Rs are phosphorylated on the cytoplasmic tail and subsequently desensitize, terminating all signaling (Faussner et al., 1998; Pizard et al., 1999). These receptors then rapidly undergo sequestration via internalization of the caveolar vesicle to the endosomal compartment where the resensitization process presumably begins (Krueger et al., 1997). Meanwhile, B_1Rs are not similarly phosphorylated and remain in caveolar membranes on the cell surface where they can continue to interact with extracellular agonist and activate MAP kinase signaling (the upstream components of this pathway are enriched in caveolae) and $cPLA_2$ activity (Xing et al., 1997; Faussner et al., 1998). Therefore, the different trafficking of these two GPCR leads to distinct signaling characteristics, especially in terms of the kinetics of the responses.

The concept of location and translocation as determinants of GPCR coupling to signal transduction pathways is a relatively new one. Recent data obtained with cardiac myocytes demonstrate that the coupling of three different endogenous receptors to a particular effector enzyme is determined primarily by the colocalization of the receptor and effector (Ostrom et al., 2001). In this case, the effector enzyme (adenylyl cyclase type 6, AC6) is almost exclusively expressed in caveolin-rich membranes. The β_1- and β_2-adrenergic receptors are significantly enriched in these same microdomains and couple to the activation of AC6, whereas a prostanoid receptor is excluded from caveolin-rich membrane fractions and cannot activate AC6 (despite its ability to activate G_s). Interestingly, the ability of β_2-adrenergic receptors to activate AC6 in these cells is diminished in comparison to β_1-adrenergic receptors. This less efficacious coupling by the β_2-adrenergic receptor is

attributed to its translocation out of caveolae upon agonist exposure (presumably to sequester in clathrin-coated pits) while the β_1-adrenergic receptors remain colocalized with AC6 in caveolae. Therefore, GPCR coupling to a given effector pathway is dependent upon both receptor-G protein coupling as well as the physical proximity of the G protein to a suitable effector.

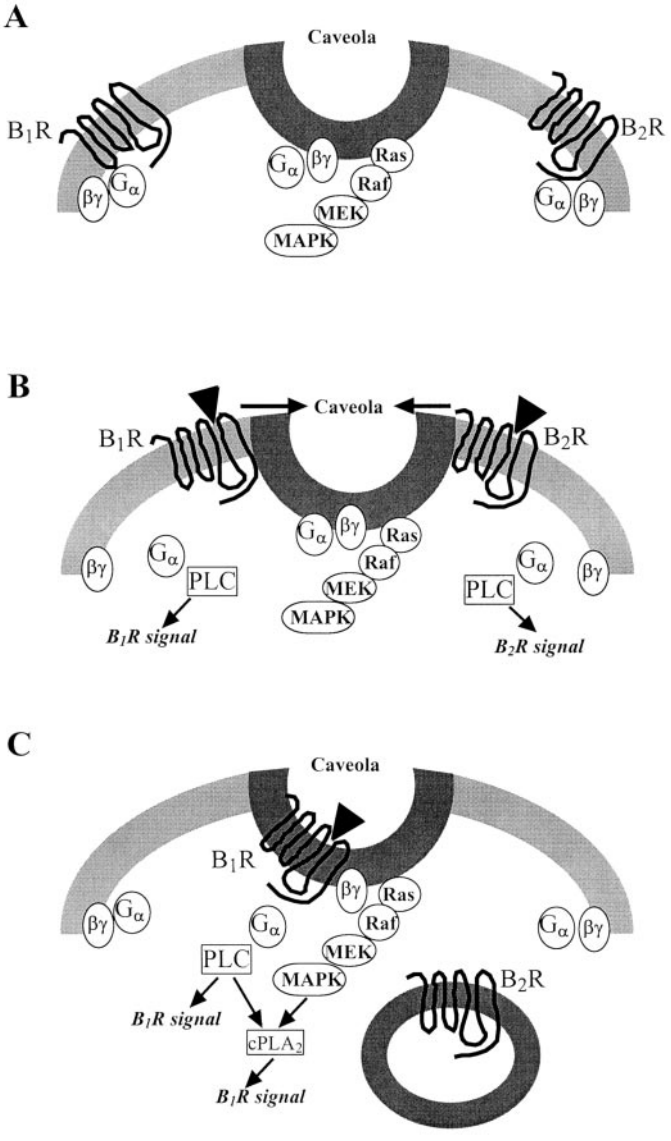

Fig. 1. Schematic diagram illustrating the localization and translocation of kinin receptors and the potential effect on their signaling. A, both kinin receptor subtypes (B_1R and B_2R) are predominantly localized in noncaveolar domains of the plasma membrane of unstimulated cells. B, upon exposure to agonist (triangles), both kinin receptor subtypes translocate to caveolin-rich domains, probably after activating the G protein and generating a signal via PLC (a membrane-associated protein) in noncaveolar domains. Since PLC and its substrate, PIP_2, are enriched in caveolae, the bulk of the signaling by both receptors likely occurs in this domain (Pike and Casey, 1996; Pike and Miller, 1998). C, the B_1R does not internalize, but remains in caveolin-rich domains where it can couple to effectors of different signaling pathways (e.g., the MAP kinase cascade) that are expressed in this location. In contrast, the B_2R internalizes rapidly, inducing only transient signals in the caveolin-rich domain. Thus, signals generated by the B_1R are more prolonged and likely result in more pronounced signaling via the effectors colocalized in caveolin-rich domains.

Key Questions

Several questions are raised by the present report by Sabourin et al. (2002) that will require further study. Do kinin receptors translocate to caveolin-rich membranes in all cells, particularly cells endogenously expressing these receptors? Is caveolar localization of kinin receptors dependent only upon protein sequence of the receptors or does it require other molecules that may be expressed in a cell-specific manner (such as caveolins or other unknown proteins)? B_2Rs can translocate to caveolae in multiple cell types, including smooth muscle (de Weerd and Leeb-Lundberg, 1997), but similar studies on endogenous B_1Rs have not been reported. What exactly are the determinants of protein localization and sequestration in caveolae? The differential state of phosphorylation of the B_2R versus the B_1R appear to be a critical determinant of kinin receptor desensitization (Faussner et al., 1998; Pizard et al., 1999), but little is known about the mechanisms regulating trafficking in caveolae. Do the B_1R and B_2R translocate to the same caveolar domains? The fact that both kinin receptors translocate to caveolae but only the B_2R undergoes internalization argues for different subpopulations of caveolae-like regions. One such region may be detergent-insoluble lipid rafts, which are present in the plasma membrane of all cells and represent domains that are biochemically similar to, but morphologically distinct from, caveolae. Some G proteins partition to rafts whereas others prefer caveolae, but the differences between these structures (other than the expression of caveolins) are not clear (Oh and Schnitzer, 2001). It is also possible that B_2Rs are only transient occupants of caveolae and internalize in a different type of vesicle, such as clathrin-coated pits (Liu et al., 1996). Do B_2Rs initiate signals that induce internalization of caveolar vesicles while B_1Rs do not? Finally, do the kinin receptors generate any signal in noncaveolar domains because PLC and PIP_2 are reportedly enriched in caveolae (Pike and Casey, 1996; Pike and Miller, 1998)? Given that PLC and PIP_2 are not completely excluded from noncaveolar membranes and the kinetics of receptor translocation are probably too slow to account for the rapidity of signal onset, it is likely that some signaling occurs before these receptors translocate to caveolae (Fig. 1). These and other questions will likely serve as the basis for investigations of kinin receptors and other GPCR in the near future.

Localization and compartmentation of the components of GPCR signal transduction represent new complexities in receptor signaling, forcing us to move beyond one-dimensional characterization of signaling pathways and consider the three-dimensional organization of these networks within the context of cell structure. A challenge for the future is to merge the evolving biochemical data with more direct morphological documentation of the fate of receptors and their various signaling partners, a challenge made more formidable by the number of molecules involved and the cell-specific, sometimes dynamic, nature of their localization.

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