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COMMENTARY

Bradykinin specificity and signaling at GPR100 and B_2 kinin receptors

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Abbreviations: AC, adenylate cyclase; BK, bradykinin; GPCR, G protein-coupled receptor; PLCβ, phospholipase Cβ

Kinins are potent and efficacious proinflammatory peptides that mediate vascular and pain responses to tissue injury. Two pharmacologically distinct kinin receptor subtypes have been identified and characterized, which are named B_1 and B_2. The B_2 receptor mediates the actions of bradykinin (BK) and Lys-BK or kallidin, the first set of bioactive kinins formed in response to injury from kininogen precursors through the actions of kallikreins, whereas the B_1 receptor mediates the actions of the kinin carboxypeptidase products desArg^{9}BK and desArg^{10}kallidin, the second set of bioactive kinins formed (Couture *et al*., 2001). Another difference between these receptors is that the B_2 receptor is ubiquitous and constitutively expressed, whereas the B_1 receptor is expressed at a very low level, if at all in healthy tissues but induced in injury by various proinflammatory cytokines such as IL-1β (Marceau *et al*., 1998). The B_1 and B_2 receptors are members of the rhodopsin family of G protein-coupled receptors (GPCR) and through Gα_q stimulate phospholipase Cβ (PLCβ) to increase phosphoinositide hydrolysis and intracellular free Ca^{2+} and through Gα_i inhibit adenylate cyclase (AC), stimulate the mitogen-activated protein kinase pathways, etc. Both B_2 and B_1 receptors are being pursued as therapeutic targets in the treatment of various inflammatory conditions and several nonpeptide ligands have been developed.

Mice lacking the B_2 receptor gene do not respond to BK (Borkowski *et al*., 1995). Thus, it was interesting when Boels & Schaller (2003) recently reported that the orphan GPCR GPR100 is a candidate BK receptor. This conclusion was based on the fact that BK, at nanomolar concentrations, increased intracellular free Ca^{2+} through GPR100 in stable CHO cells cotransfected with the promiscuous G protein Gα_{16}. GPR100 shows a relatively low level of homology (27%) with the B_2 receptor. However, this level is only marginally lower than that between the B_2 and B_1 receptor subtypes (36%). Thus, GPR100 could potentially be a kinin receptor subtype and BK a GPR100 ligand. However, this theory was questioned when Liu *et al*. (2003) almost simultaneously proposed that insulin-like peptide relaxin 3 is a ligand for GPR100 since it binds to and stimulates GTPγS binding and inhibits AC through this receptor at nanomolar concentrations. Consequently, this led to the question: Is GPR100 a BK or relaxin receptor or both?

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In this issue of the *British Journal of Pharmacology*, Meini *et al*. further investigates the pharmacology of GPR100 by directly comparing the agonist specificity of human GPR100 and the human B_2 receptor stably expressed in CHO cells. Several intriguing observations were made by these investigators as a result of this study both in terms of the ligand specificity of GPR100 as well as the mode by which agonists activate GPCR signals. First, neither BK nor relaxin 3 stimulated PLCβ activity through GPR100 in contrast to the B_2 receptor through which BK readily activated this response. Thus, the Ca^{2+} signal observed by Boels & Schaller (2003) most likely was the result of cotransfection with Gα_{16}, which is known to artificially couple transfected receptors to PLCβ. On the other hand, both agonists inhibited AC activity through GPR100. However, the BK potency ($pEC_{50} = 6.6$) was significantly lower than that of relaxin 3 and about two orders of magnitude lower than that at the B_2 receptor ($pEC_{50} = 8.6$). Furthermore, only very limited if any specific [^{3}H]BK binding to GPR100 was detected. Thus, BK is most likely not an endogenous ligand for GPR100 since the concentrations of BK required to bind and activate GPR100 would probably never be reached *in vivo*. Interestingly, BK action through GPR100 was antagonized by the BK peptide antagonist icatibant or HOE140, indicating a relationship not only in the overall sequence of GPR100 and the B_2 receptor but also specifically in their agonist-binding sites. Therefore, further analysis of the relationship between BK and relaxin 3 binding to GPR100 is warranted.

Another novel finding in this study relates to the way different agonists activate B_2 receptor signals. As expected, both BK and FR190997, a nonpeptide B_2 receptor agonist, stimulated PLCβ and inhibited AC through this receptor. Icatibant effectively antagonized PLCβ stimulation by both agonists. BK-promoted inhibition of AC was also antagonized by icatibant. On the other hand, the FR190997 response was not blocked. This surprising result suggests that the activation of Gα_i can be accomplished through two different agonist-activated receptor modes, one that is favored by BK and blocked by icatibant and another that is favored by FR190997 and insensitive to icatibant. The authors have previously shown that BK and FR190997 utilize distinct B_2 receptor-binding epitopes (Bellucci *et al*., 2003), but it is generally thought that such different binding modes translate into a common coupling mode to a specific G protein. The differential sensitivity of BK and FR190997 to icatibant

suggests otherwise. At GPCRs that couple to multiple effectors, agonists have been found that selectively activate certain effectors. This event, called agonist-directed trafficking of receptor signals, is thought to be due to the fact that agonists select for different receptor conformational states with preference for different G proteins (Kenakin, 2001). The observation reported here extends this theory to indicate that the coupling to a specific G protein involves more than one receptor state each further discriminated by certain ligands. That the coupling to the two different G proteins $G\alpha_i$ and $G\alpha_q$ involves unique B_2 receptor states, as expected in the above model, was shown by the completely different icatibant sensitivity of FR190997-promoted stimulation of PLCβ and inhibition of AC. The use of synthetic ligands has clearly provided a plethora of data on the details of GPCR activation and G protein coupling. If harnessed, such information should greatly expand the avenues by which drugs may be designed to modulate these therapeutically highly valuable receptors.

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