0026-895X/02/6202-281–288$7.00
MOLECULAR PHARMACOLOGY
Copyright © 2002 The American Society for Pharmacology and Experimental Therapeutics
*Mol Pharmacol* 62:281–288, 2002

Vol. 62, No. 2
1699/994958
*Printed in U.S.A.*



# Negative and Positive Regulatory Epitopes in the C-Terminal Domains of the Human B1 and B2 Bradykinin Receptor Subtypes Determine Receptor Coupling Efficacy to $G_{q/11}$-Mediated Phospholipase C$\beta$ Activity

DONG SOO KANG and L. M. FREDRIK LEEB-LUNDBERG

*Department of Biochemistry, The University of Texas Health Science Center, San Antonio, Texas*

Received February 26, 2002; accepted April 16, 2002

This article is available online at http://molpharm.aspetjournals.org

## ABSTRACT

The human B1 bradykinin (BK) receptor (B1R) is more efficacious than the human B2 BK receptor (B2R) in both ligand-independent and agonist-dependent coupling to $G_{q/11}$-mediated phospholipase C$\beta$ activity. In fact, B1R is constitutively active, whereas B2R exhibits little if any constitutive activity. To evaluate the role of the C-terminal domain in receptor $G_{q/11}$ coupling, we constructed chimeric and C-terminally truncated receptors. The slopes of the increase in basal and agonist-dependent cellular phosphoinositide hydrolysis as a function of receptor density in transiently transfected human embryonic kidney 293 cells provided parameters of receptor coupling. Exchanging the C-terminal domains between the two receptors revealed that these domains are largely responsible for the difference in coupling. B1R truncation showed that this receptor does not directly depend on the C-terminal domain for efficient coupling, although coupling is dramatically augmented by residues in the membrane-distal portion of the domain downstream from $Tyr^{327}$. On the other hand, coupling of B2R is absolutely dependent on a membrane-proximal epitope in the C-terminal domain upstream from $Lys^{315}$. This epitope is adjacent to a basic residue, $Arg^{311}$, which exerts an inhibitory effect on coupling. $Arg^{311}$ is not conserved in B1R, and complementary mutations in B2R and B1R showed that this residue, together with previously identified serines and threonines, acts to attenuate the coupling efficacy of B2R. Therefore, the C-terminal domain participates intimately in the efficacy of B1R and B2R $G_{q/11}$ coupling by contributing both positive and negative regulatory epitopes.

The B1 and B2 receptors are receptor subtypes for kinins, which are proinflammatory peptides and among the most potent and efficacious vasodilator agonists known (Bhoola et al., 1992). These receptors are prototypical members of the rhodopsin family of heptahelical receptors, albeit exhibiting a relatively low level of homology (36%) (Hess et al., 1992; Menke et al., 1994). The B2 receptor mediates the actions of bradykinin (BK) and Lys-BK or kallidin (KD), the first set of bioactive kinins formed in response to injury from kininogen precursors through the actions of kallikreins, whereas the B1 receptor mediates the actions of the kinin carboxypeptidase products $desArg^{9}$BK and $desArg^{10}$KD, the second set of bioactive kinins formed (Regoli and Barabe, 1980). Evidence was recently presented that the human B2 receptor may also bind and be activated directly by kallikrein and some other serine proteases (Hecquet et al., 2000). Like kinin production, receptor subtype expression is also sequential because the B2 receptor is constitutively expressed, whereas the B1 receptor is expressed at a very low level, if at all, in healthy tissues but induced in injury by proinflammatory cytokines (Marceau et al., 1998) as well as by kinins themselves (Schanstra et al., 1998; Phagoo et al., 1999).

The rationale for constitutively expressing the B2 receptor but restricting B1 receptor expression primarily to conditions of injury is not understood. Both receptors couple in a pertussis toxin-insensitive manner (Tropea et al., 1993) through $G_{q/11}$ (Gutowski et al., 1991; Jones et al., 1995; Austin et al., 1997) to phospholipase C$\beta$ activity and subsequent phosphoinositide (PI) hydrolysis. Additional and/or sequential activation of other $Ca^{2+}$-dependent and -independent phospholipase C isoforms is possible. The participation of $G_q$ versus $G_{11}$ has been defined for few if any receptors, but the two are thought to be very similar and overlap in function (Offermanns et al., 1998). Despite both coupling through $G_{q/11}$, the two receptors exhibit rather different signaling patterns, which may explain their distinct and sequential patterns of expression. The B1 receptor is significantly more active than the B2 receptor in stimulating PI hydrolysis both in terms of

This work was supported by National Institutes of Health grant GM41659.

**ABBREVIATIONS:** BK, bradykinin; KD, kallidin; PI, phosphoinositide; WT, wild-type; ICIV, fourth intracellular domain; B1R, human B1 bradykinin receptor; B2R, human B2 bradykinin receptor; HEK, human embryonic kidney; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethane-sulfonic acid.

An erratum has been published:
http://molpharm.aspetjournals.org/content/62/3/762.full.pdf

ligand-independent, spontaneous activity and agonist-dependent activity (Leeb-Lundberg et al., 2001). Indeed, the B1 receptor is constitutively active, whereas the B2 receptor exhibits little if any activity in the absence of agonist ligands (Leeb-Lundberg et al., 2001). Furthermore, the B1 receptor desensitizes and sequesters slowly upon activation, whereas the B2 receptor desensitizes and sequesters rapidly (Mathis et al., 1996; Lamb et al., 2001). These differences are probably critical to the specific roles of these receptors in the various stages of the inflammatory and pain response to injury (Couture et al., 2001).

Current models assert that heptahelical receptors spontaneously isomerize between inactive and activated conformational states termed R and R*, respectively, and that R* associates with a G protein to form R*G, which triggers the intracellular signal (Samama et al., 1993). In this model, agonists act by favoring or stabilizing R*. Isomerization is thought to involve movements within the helical bundle of the receptor (Gether, 2000; Marie et al., 2001), which translate into a series of events involving receptor G protein binding and activation. The latter events are generally referred to collectively as G protein coupling because they cannot be effectively discriminated (Wess, 1997). In this report, we use the term "coupling efficacy" to portray the difference in the extent of coupling between different receptors and regulatory conditions.

G protein coupling and selectivity is mediated through the intracellular receptor domains (Wess, 1998). No single common G protein-coupling epitope has been identified in these receptors, even though the DRY sequence at the bottom of the third transmembrane helix may be of general importance in the receptor-triggered G protein activation process (Scheer et al., 1996). The contribution of the receptor C-terminal domain to G protein coupling seems to vary with the receptor and the G protein (Wess, 1998). However, this domain has been shown to directly influence receptor coupling efficacy (Claeysen et al., 1999). This effect may in part be linked to the presence of epitopes in this domain that are involved in receptor desensitization (Krupnick and Benovic, 1998; Pitcher et al., 1998). Indeed, we reported recently that a cluster of serines and threonines in the B2 receptor C-terminal domain, which is important for receptor phosphorylation (Blaukat et al., 1999, 2001), internalization (Pizard et al., 1999), and desensitization (Fathy et al., 1999), is at least partially responsible for the lower coupling efficacy of this receptor subtype relative to that of the B1 receptor subtype (Fathy et al., 1999; Leeb-Lundberg et al., 2001).

In the present study, we have analyzed in more detail the roles of the B1 and B2 receptor C-terminal domains in coupling to $G_{q/11}$-mediated phospholipase C$\beta$ activation. By doing so, we have identified additional negative and positive regulatory epitopes in the C-terminal domains that determine the coupling efficacies of these receptors.



## Experimental Procedures

**Materials.** 2,3-Prolyl-3,4-[$^3$H]bradykinin (90–114 Ci/mmol), des(Arg$^{10}$)-3,4-prolyl-3,4-[$^3$H]kallidin (64–107 Ci/mmol), and [*myo*-$^3$H]inositol (10–20 Ci/mmol) were obtained from PerkinElmer Life Sciences (Boston, MA). M2 monoclonal antibodies against the FLAG epitope were obtained from Kodak IBI (New Haven, CT) and Sigma-Aldrich (St. Louis, MO). DesArg$^{10}$kallidin and bradykinin were purchased from Bachem California (Torrance, CA). The original human and rabbit B1 receptor and human B2 receptor cDNAs were obtained from F. Hess (Merck Research Labs, West Point, PA). All other chemicals were obtained as described previously (Fathy et al., 1999; Leeb-Lundberg et al., 2001).

**Mutation and Transfection.** Mutations were done using a polymerase chain reaction-ligation-polymerase chain reaction protocol as described previously (Fathy et al., 1999; Leeb-Lundberg et al., 2001). The FLAG epitope was inserted at the receptor N terminus immediately after the initial methionine. Human embryonic kidney 293 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated horse serum in 10% $CO_2$ at 37°C. The cells were transiently transfected with varying amounts of DNA using the calcium phosphate precipitate method as described previously (Fathy et al., 1999; Leeb-Lundberg et al., 2001).

**Particulate Preparation.** Transfected HEK293 cells were washed twice with ice-cold phosphate-buffered saline and then pelleted by centrifugation at 2,000*g* for 10 min. The cells were then resuspended in a buffer containing 25 mM TES, pH 6.8, 0.5 mM EDTA, 0.2 mM $MgCl_2$, and 1 mM 1,10-phenanthroline and homogenized using a T25 Ultra-Turrax tissue homogenizer (IKA Works, Inc., Wilmington, NC) at 20,500 rpm for 10 s. Membranes were isolated by centrifugation at 45,000*g* for 30 min at 4°C and washed 1 to 3 times in the above buffer depending on the experiment. The pellets were then resuspended in the same buffer supplemented with 0.1% bovine serum albumin and 0.014% bacitracin (binding buffer) and used immediately.

**Radioligand Binding.** Radioligand binding assays were performed essentially as described previously (Leeb-Lundberg et al., 2001). Receptor density was determined on intact HEK293 cells by incubating cells in Leibovitz's L-15 medium, pH 7.4, 0.1% bovine serum albumin including the protease inhibitors bacitracin (140 $\mu$g/ml) and 1,10-phenanthroline (1 mM), and a saturating concentration of [$^3$H]desArg$^{10}$KD or [$^3$H]BK (3–5 nM) at 4°C for 60 to 90 min. Competition binding was done on particulate preparations by incubating the preparations in binding buffer including approximately 0.2 to 0.5 nM [$^3$H]desArg$^{10}$KD or [$^3$H]BK with and without various concentrations of competitor at 25°C for 60 to 90 min.

**Receptor Activity.** Activities of various receptor constructs were assayed by monitoring PI hydrolysis in HEK293 cells transfected with a series of receptor cDNA levels and labeled with 1 $\mu$Ci/ml [*myo*-$^3$H]inositol as described previously (Fathy et al., 1999; Leeb-Lundberg et al., 2001). After washing, the cells were incubated in Leibovitz's L-15 medium containing 5 mM LiCl in the absence and presence of an agonist or antagonist for 30 min at 37°C. The slope factors of the increase in basal cellular PI hydrolysis and agonist-dependent PI hydrolysis as a function of the level of receptor expression, which we term index of basal receptor activity, or $I_B$, and index of agonist-stimulated receptor activity, or $I_A$, respectively, were used as parameters of receptor activity.

**Immunoprecipitation and Immunoblotting.** HEK293 cells transfected with FLAG epitope-tagged receptors were subjected to immunoprecipitation and immunoblotting essentially as described previously (Leeb-Lundberg et al., 2001). In short, cells were solubilized in lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 10 mM NaF, 10 mM sodium phosphate, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, 10 $\mu$g/ml leupeptin, and 0.1 mM phenylmethylsulfonyl fluoride) for 30 min at 4°C. The lysate was centrifuged at 13,000*g* for 15 min at 4°C. The supernatant (1 ml) was then incubated 12 to 18 h with anti-FLAG M2 antibody (1:200) followed by incubation with Protein A-Sepharose beads precoupled to rabbit anti-mouse IgG for an additional 2 h at 4°C. The beads were then washed with 2 × 1 ml of lysis buffer and then with 1 ml of 10 mM Tris-HCl, pH 7.4. The pellet was heated in SDS-polyacrylamide gel electrophoresis buffer containing 6% $\beta$-mercaptoethanol for 5 min at 100°C and then electrophoresed on 12% polyacrylamide gels. The gel was then electroblotted onto 0.45-$\mu$m nitrocellulose membranes and stained with anti-FLAG M2 antibody (1:1000). Immunoreactive

bands were visualized with an immunodetection kit using peroxidase-labeled sheep anti-mouse antibody according to the procedure described by the supplier (PerkinElmer Life Sciences).

**Data Analysis.** Where indicated, data are presented as the mean ± S.E. and were compared using the Student's *t* test. The ligand binding constant, $K_i$, for the various receptor constructs was calculated by the Radlig program (Biosoft, Ferguson, MO) using data from radioligand competition binding experiments as described above.

## Results

**The C-Terminal Domains of the Human B1 and B2 Receptor Subtypes Participate in Regulating Receptor Coupling Efficacy.** The difference in $G_{q/11}$ coupling efficacy between the human B1 and B2 receptors is clearly reflected in the slope factors of the increase in basal cellular PI hydrolysis (Fig. 1A; Table 1) and agonist-dependent PI hydrolysis (Fig. 1B; Table 1) as a function of the level of B1 and B2 receptor expression in HEK293 cells. These factors, which we term index of basal receptor activity, or $I_B$, and index of agonist-stimulated receptor activity, or $I_A$, respectively, provide convenient parameters of receptor coupling because they are normalized for the level of receptor expression. The contribution of the fourth intracellular (ICIV), C-terminal domains to this difference is clear from the fact that substitution of the B2 receptor domain in the B1 receptor at the conserved residues $Gly^{316}$ (B1) and $Gly^{309}$ (B2) to make B1(B2ICIV) inhibited $I_B$ and $I_A$ 98 and 84%, respectively, and consequently rendered this chimera without any significant constitutive activity (Fig. 1, A and B; Table 1). This decrease is in part due to the presence of a cluster of serines and threonines specifically in the B2 receptor C-terminal domain, including $Ser^{339}$, $Thr^{342}$, $Thr^{345}$, $Ser^{346}$, and $Ser^{348}$, that is phosphorylated (Blaukat et al., 2001) and critical for B2 receptor desensitization (Fathy et al., 1999). Indeed, Ala mutation of this cluster to make B1(B2ICIV$A^{Ser/Thr}$) and B1(B2ICIV$A^{Ser/Thr}$)$A^{346}$ ($Ser^{346}$ in the chimera corresponds to $Ser^{339}$ in the WT B2 receptor) partially rescued both B1 receptor activities (Table 1) (Leeb-Lundberg et al., 2001). In contrast, substitution of the B1 receptor C-terminal domain in the B2 receptor increased $I_A$ 413%, providing further evidence for the negative regulatory effect of the B2 receptor domain but possibly also for positive regulatory epitopes in the B1 receptor domain. The fact that this substitution did not afford the B2 receptor with constitutive activity indicates that the Ser/Thr cluster is not the only determinant of the coupling efficacy of these receptor subtypes. Accordingly, further structure-function studies of these domains were pursued.

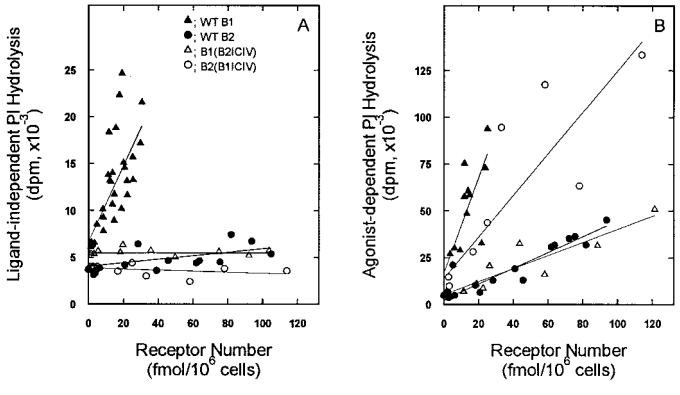


**Fig. 1.** Ligand-independent and agonist-dependent PI hydrolysis by the human WT B1 and B2 receptors and receptor chimeric mutants. HEK293 cells transfected with varying amounts of the WT B1 (▲) and WT B2 (●) receptors and the chimeric receptor mutants B1(B2ICIV) (△) and B2(B1ICIV) (○) were incubated in the absence (A) and presence (B) of either 1 μM desArg$^{10}$KD [WT B1, B1(B2ICIV)], or 1 μM BK [WT B2, B2(B1ICIV)] as indicated and then assayed for PI hydrolysis and radioligand binding as described under *Experimental Procedures*. Note the difference in the *y*-axis scale in A and B. The results are from 4 to 8 independent experiments with each point performed in duplicate.

**Differential Contribution of the Human B1 and B2 Receptor C-Terminal Domains to Receptor Coupling Efficacy.** To analyze in more detail functional elements in the B1 and B2 receptor C-terminal domains, we created a series of C-terminal truncation mutants of each receptor subtype (Fig. 2A). Truncation had no significant effects on the binding affinities of either the B1 agonist desArg$^{10}$KD or the B2 agonist BK (Table 1). Immunoblotting of N-terminally FLAG-tagged B1 receptor constructs was used to identify the receptor truncation mutants and show that the mutations did not significantly alter total receptor expression (Fig. 2B). Technical difficulties prevented the use of FLAG-tagged B2 receptor constructs.

Figure 3 and Table 1 display the activity indices $I_A$ and $I_B$ for the WT and truncated B1 receptors. Successive deletion from the receptor C-terminal end of 8, 17, and 26 amino acid residues to yield the constructs B1Stop346, B1Stop337, and B1Stop328 decreased $I_A$ 23, 59, and 84%, respectively, and $I_B$ 42, 45, and 72%, respectively. Truncation beyond $Tyr^{327}$ to yield B1Stop323, B1Stop322, B1Stop321, and B1Stop320 did not significantly decrease further either $I_A$ or $I_B$ of the B1 receptor, indicating that this receptor is able to couple to $G_{q/11}$ and phospholipase Cβ in the absence of almost the entire C-terminal domain. Indeed, the coupling of the most truncated B1 receptor mutant (B1Stop320) was as efficacious as that of the WT B2 receptor (Table 1). Still, coupling of the B1 receptor is greatly enhanced by an epitope downstream from $Tyr^{327}$. That this epitope does not introduce additional coupling to $G_{i/o}$-like proteins, which could potentially contribute to stimulation of PI metabolism through the release of βγ, was evident from the complete lack of sensitivity of both the ligand-independent and agonist-dependent B1 receptor response to pertussis toxin (data not shown). Thus, the C-terminal domain of the B1 receptor seems to contribute primarily positive regulatory epitopes for $G_{q/11}$ coupling.

In contrast to the B1 receptor, B2 receptor-mediated PI hydrolysis was highly dependent on the length of the C-terminal domain. Furthermore, the pattern of sensitivity was rather different and considerably more complex than that of the B1 receptor (Fig. 4; Table 1). In addition, no ligand-independent activity was detected in any of the B2 receptor truncation mutants. Deletion from the receptor C-terminal end of 44 amino acid residues yielded a construct, B2Stop321, which exhibited 10% of the activity of the WT receptor (Fig. 4; Table 1). Deletion of only one additional residue, $Tyr^{320}$, to yield B2Stop320 resulted in a complete recovery of receptor activity. The reduced activity of B2Stop321 may be due to the exposure of $Tyr^{320}$, which has been proposed to serve a regulatory role in this receptor (Prado et al., 1997). B2Stop316 and B2Stop315, in which additional five and six residues, respectively, had been deleted, remained fully active. On the other hand, removal of only one more residue, $Lys^{314}$, to yield B2Stop314 led to a complete loss of receptor activity, and the

TABLE 1

Agonist binding constants ($K_i$) and ligand-independent ($I_B$) and agonist-dependent ($I_A$) coupling efficacy of human WT and mutant B1 and B2 receptors in HEK293 cells

$K_i$ values were obtained from competition binding experiments on intact HEK293 cells expressing each receptor construct and calculated using the Radlig curve-fitting program. The values are presented as the average ± S.E. of at least three experiments or the average of two experiments as indicated after the value. $I_A$ and $I_B$ values are the slopes from experiments in which cellular PI hydrolysis (dpm) in the absence and presence of agonist was determined as a function of the receptor number (fmol/$10^6$ cells) as described in Fig. 1. Values were generated with points from at least three different experiments, or two experiments as indicated after the value, with each experiment yielding at least five points and with each point done in duplicate.

| Construct | $K_i$ | | $I_B$ | $I_A$ |
|---|---|---|---|---|
| | desArg$^{10}$KD | BK | | |
| | *nM* | | | |
| WT B1 | 1.67 ± 0.27 | UB | 198 | 2058 |
| B1Stop346 | 2.90 ± 0.90 | UB | 114 | 1580 |
| B1Stop337 | 2.28 ± 0.72 | UB | 108 | 840 |
| B1Stop328 | 5.70 ± 1.15 | UB | 55 | 320 |
| B1Stop323 | | | 37 | 289 |
| B1Stop322 | 3.24 (2) | UB | 11 | 320 |
| B1Stop321 | 2.15 (2) | UB | 48 | 404 |
| B1Stop320 | 4.02 ± 0.24 | UB | 23 | 425 |
| B1R$^{318}$ | 0.194 ± 0.031 | UB | 198 | 908 |
| WT B2 | UB | 1.86 ± 0.34 | 0 | 194 |
| B2Stop321 | UB | 2.05 ± 0.60 | 0 | 20 |
| B2Stop320 | UB | 1.06 ± 0.75 | 0 | 251 |
| B2Stop316 | UB | 0.940 | 0 | 177 |
| B2Stop315 | | | 0 | 151 |
| B2Stop314 | UB | 1.79 ± 0.28 | 0 | 0 |
| B2Stop313 | UB | 3.37 ± 0.77 | 0 | 2 |
| B2A$^{311}$ | UB | 1.09 ± 0.11 | 22 | 1256 |
| B2A$^{314}$ | | | 0 | 579 |
| B2A$^{324}$ | | | 0 | 392 |
| B2A$^{329}$ | | | 0 | 244 |
| B2Stop313A$^{311}$ | | | 0 | 41 (2) |
| B2Stop321A$^{311}$ | UB | 1.09 ± 0.11 | 0 | 34 (2) |
| B2Stop315A$^{314}$ | | | 2 | 265 (2) |
| B1(B2ICIV) | 2.53 ± 0.23 | | 4 | 337 |
| B1(B2ICIV)Stop320 | | | 4 | 90 |
| B1(B2ICIV)Stop320A$^{317}$ | | | 15 | 108 (2) |
| B1(B2ICIV)Stop320A$^{318}$ | | | 43 | 562 |
| B1(B2ICIVA$^{Ser/Thr}$) | 4.04 ± 0.24 | | 40 | 834 |
| B1(B2ICIVA$^{Ser/Thr}$)A$^{346}$ | | | 122 | 982 |
| B2(B1ICIV) | | | 0 | 995 |

UB, undetectable binding of the radioligand to the construct.

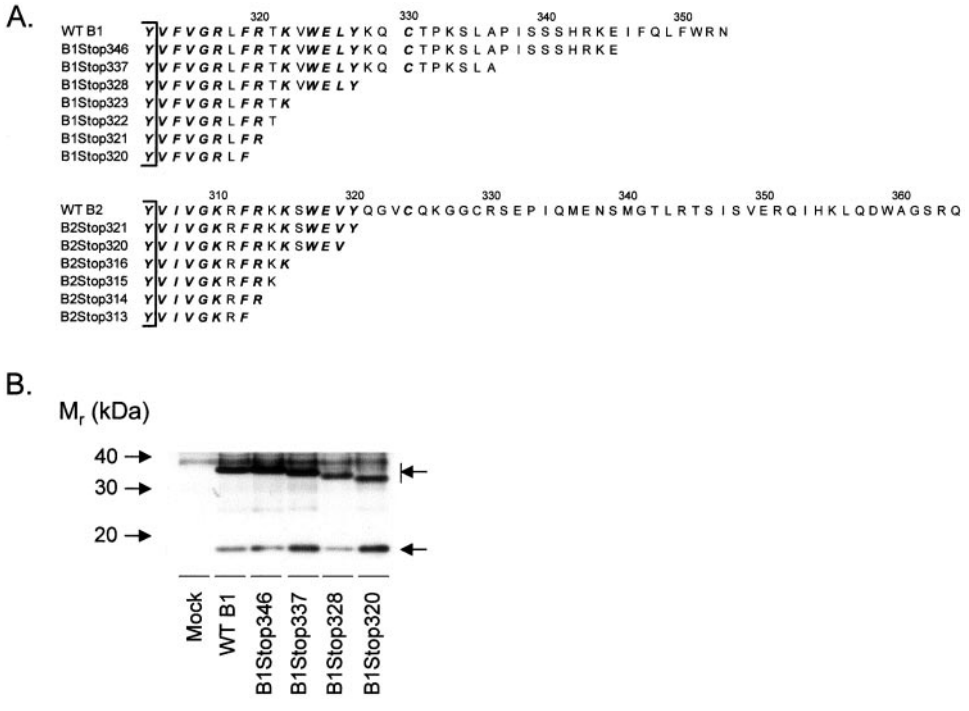


**Fig. 2.** A, amino acid sequences of the C-terminal domains of the human WT and truncated B1 and B2 receptors and the expression of FLAG-tagged B1 receptor constructs. Indicated is the predicted junction of the seventh transmembrane domain and the C-terminal domain (half bracket) (Palczewski et al., 2000) and conserved residues between the receptor subtypes (bold italics). The numbering of residues in the B1 and B2 receptors is according to Menke et al. (1994) and Hess et al. (1992), respectively. B, HEK293 cells transfected with N-terminally FLAG-tagged derivatives of WT B1, B1Stop346, B1Stop337, B1Stop328, and B1Stop320 were immunoprecipitated and immunoblotted with anti-FLAG antibodies as described under *Experimental Procedures*. Indicated are the molecular mass standards (left side arrows) and the receptor peptides (right side arrows). The smaller molecular mass receptor peptide is thought to be a FLAG-tagged proteolytic product of the receptor. The results are from a representative experiment performed three times.

receptor remained inactive by further truncation to make B2Stop313. Replacement of Lys$^{314}$ with Ala to make B2Stop315A$^{314}$ yielded a fully functional construct, indicating that it was the length rather the specific presence of Lys at this position that was important for coupling (Table 1). These results show that the B2 receptor C-terminal domain participates in a rather complex way in receptor function, apparently contributing both structural and negative regulatory epitopes for $G_{q/11}$ coupling.

**Identification of a Basic Residue in the C-terminal Domain of the Human B2 Receptor That Inhibits Receptor Coupling Efficacy.** Substitution of the B2 receptor C-terminal domain in the B1 receptor to make B1(B2ICIV) led to decreases in $I_B$ and $I_A$ of 98 and 83%, respectively (Table 1). Deletion of the C-terminal 52 amino acid residues in this chimera to make B1(B2ICIV)Stop320 further decreased $I_A$ 73% compared with B1(B2ICIV) (Table 1). This truncated construct differs from B1Stop320 by only two residues, which are those at positions 317 and 318 (Fig. 2A). In other words, B1Stop320 terminates in the B1-specific sequence Arg-Leu-Phe, whereas B1(B2ICIV)Stop320 terminates in the B2-specific sequence Lys-Arg-Phe. Despite this minor difference, the latter construct exhibited $I_B$ and $I_A$ values of 83 and 79%, respectively, lower than those of the former construct (Fig. 5A; Table 1). To identify the residue responsible for this difference, we substituted positions 317 and 318 with either Ala-Arg or Lys-Ala. As shown in Fig. 5A and Table 1, substitution with Ala-Arg led to $I_B$ and $I_A$ values similar to those in the presence of Lys-Arg. On the other hand, substitution with Lys-Ala yielded $I_B$ and $I_A$ values similar to those in the presence of Arg-Leu. Clearly, it is the presence of a basic residue at position 318 that renders B1(B2ICIV)Stop320 less active than B1Stop320. Interestingly, this is the only nonconserved position in a stretch of eight residues following the NP*X*X*Y* motif in the seventh transmembrane domains of the B1 and B2 receptors (Fig. 2A).

As the above-described basic residue is native only to the B2 receptor, it is in the milieu of this receptor that its functional role is best evaluated. To this end, we mutated Arg$^{311}$ to Ala in the WT B2 receptor (B2A$^{311}$). As shown in Fig. 5B and Table 1, this mutation resulted in a 550% increase in $I_A$. Furthermore, the receptor gained some ligand-independent activity. On the other hand, Ala mutation of Lys$^{314}$ (B2A$^{314}$), Cys$^{324}$ (B2A$^{324}$), and Cys$^{329}$ (B2A$^{329}$) only had small effects on the receptor (Table 1). Obviously, Arg$^{311}$ exerts a relatively specific inhibitory effect on the coupling efficacy of the B2 receptor. Indeed, substitution of this residue at the corresponding position in the B1 receptor, which is occupied by a leucine, to make B1R$^{318}$ decreased $I_A$ 56% (Table 1). Thus, more or less complementary mutations at this position in the B2 receptor (Arg$^{311}$ → Ala) and B1 receptor (Leu$^{318}$ → Arg) effectively eliminated the difference in the agonist-dependent

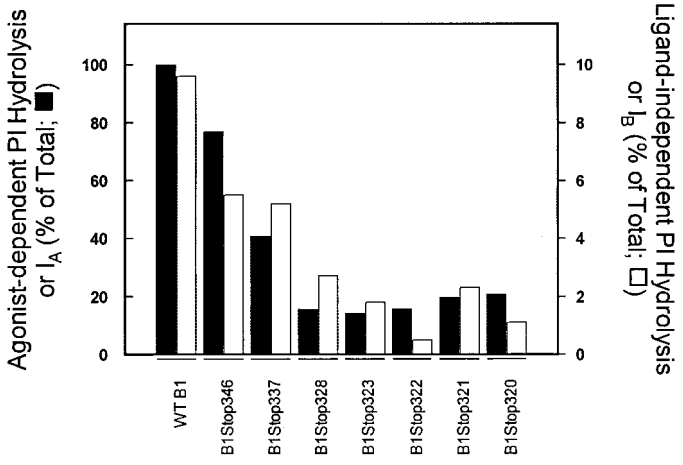


**Fig. 3.** Ligand-independent and agonist-dependent PI hydrolysis by the human WT and truncated B1 receptors. HEK293 cells transfected with varying amounts of WT B1, B1Stop346, B1Stop337, B1Stop328, B1Stop323, B1Stop322, B1Stop321, and B1Stop320 were incubated in the absence (open bars) and presence (filled bars) of 1 $\mu$M desArg$^{10}$KD as indicated and then assayed for PI hydrolysis and radioligand binding as described under Fig. 1. The results are presented as percent of total where 100% total is the slope index $I_A$ of PI hydrolysis in cells expressing the WT B1 receptor in the presence of 1 $\mu$M desArg$^{10}$KD. Note the difference in the scales of the two axes and that the left *y*-axis refers to the filled bars and the right *y*-axis refers to the open bars. Slopes were generated with points from at least three experiments, with each experiment yielding at least five points and each done in duplicate.

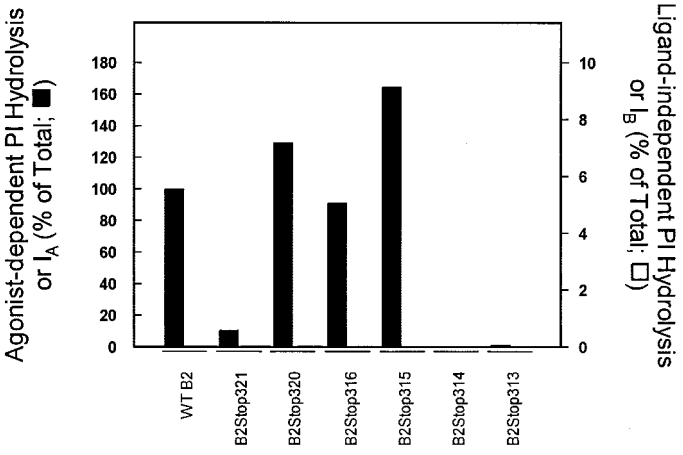


**Fig. 4.** Ligand-independent and agonist-dependent PI hydrolysis by the human WT and truncated B2 receptors. HEK293 cells transfected with varying amounts of WT B2, B2Stop321, B2Stop320, B2Stop316, B2Stop315, B2Stop314, and B1Stop313 were incubated in the absence (open) and presence (filled) of 1 $\mu$M BK as indicated and then assayed for PI hydrolysis and radioligand binding as described under Fig. 1. For further details, see legend to Fig. 3. Note that the values for the open bars are too small to be detected in the figure.

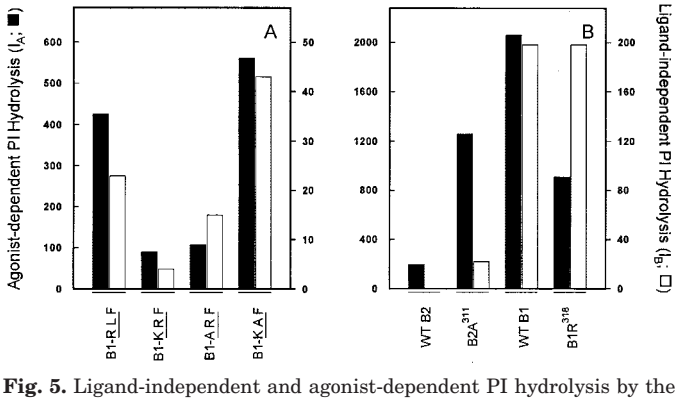


**Fig. 5.** Ligand-independent and agonist-dependent PI hydrolysis by the human WT and mutated B1 and B2 receptors. HEK293 cells transfected with varying amounts of the B1 truncation mutants B1Stop320 (B1-RLF), B1(B2ICIV)Stop320 (B1-KRF), B1(B2ICIV)Stop320A$^{317}$ (B1-ARF), and B1(B2ICIV)Stop320A$^{318}$ (B1-KAF) (A) and WT B2, B2A$^{311}$, WT B1, and B1R$^{318}$ (B) as indicated. In both A and B, the cells were incubated in the absence (open bars) and presence (filled bars) of 1 $\mu$M desArg$^{10}$KD and then assayed for PI hydrolysis and radioligand binding as described under Fig. 1. For further details, see legend to Fig. 3.

coupling efficacies of the two receptor subtypes. Interestingly, $I_B$ of the B1 receptor did not decrease by the $Leu^{318} \rightarrow$ Ang mutation, providing some evidence that the structural basis for the ligand-independent and agonist-dependent activities of the B1 receptor may be different. However, the complementary mutations reduced the difference in the ligand-independent activities because that of the B2 receptor increased slightly upon the $Arg^{311} \rightarrow$ Ala mutation.

We then asked ourselves if $Arg^{311}$ could be responsible for the lack of activity of B2Stop313. To address this question, we mutated $Arg^{311}$ to Ala in this truncation mutant ($B2Stop313A^{311}$). Interestingly, this construct remained essentially inactive (Table 1). Indeed, even B2Stop321 was virtually insensitive to Ala mutation of this residue (Table 1). Thus, $Arg^{311}$ seems to require an epitope(s) downstream from $Tyr^{320}$ to inhibit B2 receptor coupling. This is in contrast to the B1 receptor, in which inhibition by Arg was independent of the length of the C-terminal domain [compare B1(B2ICIV)Stop320 and $B1(B2ICIV)Stop320A^{318}$ in Fig. 5A and Table 1].

## Discussion

Our interest in the B1 and B2 receptor C-terminal domains in terms of receptor $G_{q/11}$ coupling stems in part from the level of structural homology of this domain between the two receptor subtypes and between the various species variants of each subtype. The human B1 and B2 receptor C-terminal domains exhibit only 22% overall conservation. Assuming that the seventh transmembrane helix ends at the Tyr in the NP*XX*Y motif in heptahelical receptors (Palczewski et al., 2000), the conservation is very high (80%) among the first 15 residues in the C-terminal domain. On the other hand, virtually no conservation exists downstream of these residues. The species conservation in the B2 receptor C-terminal domain is relatively high (≥71%), which is in contrast to the B1 receptor domain, where the rodent B1 receptors lack the 26 most C-terminal residues of the human, dog, and rabbit receptors.

Based on the lack of species conservation in the B1 receptor C-terminal domain, it is not surprising that the human B1 receptor couples to $G_{q/11}$ with only seven residues (residues 313–319) in this domain. Indeed, the human receptor mutant corresponding to the rodent WT B1 receptors, which is B1Stop328, exhibits an activity that is almost the same as that of the human WT B2 receptor. Hence, the B1 receptor may have adopted a mechanism of $G_{q/11}$ coupling that does not directly require the C-terminal domain. The human receptor is by no means independent of this domain because the distal 26 residues beyond $Tyr^{327}$ greatly augment the receptor response. Given that these additional residues are absent in the rodent receptors, they probably serve a regulatory rather than a direct structural role in $G_{q/11}$ coupling.

In contrast to the B1 receptor, seven C-terminal residues (residue 306–312) were not sufficient for the B2 receptor to stimulate $G_{q/11}$-mediated phospholipase C$\beta$ activity. In fact, stepwise addition of downstream residues revealed that this receptor was absolutely dependent on nine residues (residue 306–314) for coupling. Interestingly, the crystal structure of bovine rhodopsin reveals that the residues in this receptor corresponding to B2 receptor residues 310 through 321 form an amphipathic $\alpha$ helix (Palczewski et al., 2000). Seven modeling programs based on either sequence algorithms or amino acid helix propensity predicted that B2 receptor residues 310 through 325 also form a helix. In the B2 receptor, a putative eighth receptor helix would start after $Gly^{309}$, a residue with high *N*-cap propensity, and end at $Gln^{325}$ and would contain numerous residues with high helix propensity that could participate in both intrahelical charge-charge and hydrophobic interactions (Chakrabartty and Baldwin, 1995). The helical wheel projection of the B2 receptor residues shown in Fig. 6 clearly defines the amphipathic character of the eighth receptor helix. Interestingly, the nine C-terminal residues required for B2 receptor activity would be the minimum number for one full turn of this helix.

Included in the putative helical region of the human B2 receptor C-terminal domain that is critical for $G_{q/11}$ coupling is a BB*X*B/BB*XX*B motif (-$Lys^{310}$-$Arg^{311}$-$Phe^{312}$-$Arg^{313}$-), which has been proposed to be important for G protein coupling in some heptahelical receptors (Okamoto and Nishimoto, 1992; Hogger et al., 1995; Lee et al., 1996). Interestingly, the rabbit, rat, mouse, and dog receptors contain two contiguous motifs in this region. This is the only such motif on the intracellular surface of the human B2 receptor, which may explain the dependence of the B2 receptor on the C-terminal domain for $G_{q/11}$ coupling. One additional residue, $Lys^{314}$, is necessary for effective coupling of the receptor, and this residue can be either Lys or Ala. The position of such a motif is conserved in the $AT_{1A}$ receptor, which is also dependent on a membrane-proximal epitope in the C-terminal domain for $G_{q/11}$ coupling (Sano et al., 1997). This receptor requires four residues beyond this motif for function, providing further evidence that the motif may have to exist in a helix. Interestingly, the B1 receptor C-terminal domain does not contain such a motif. Although the two receptor subtypes are highly conserved in this region, the above motif is dis-

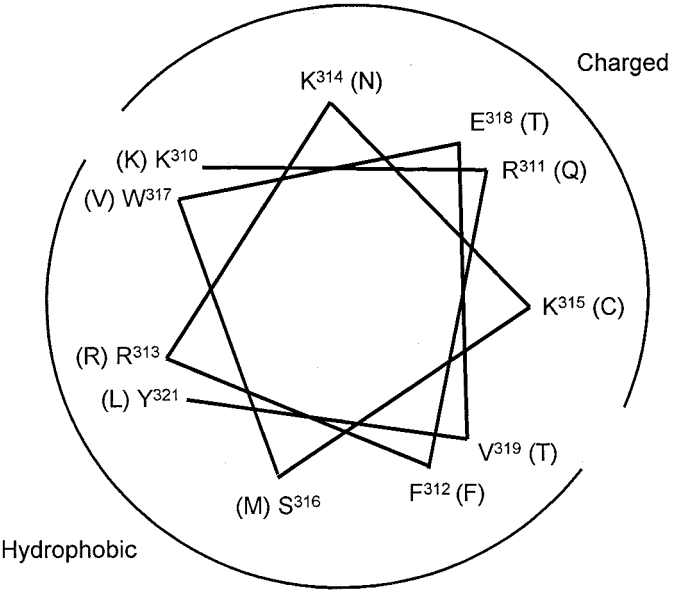


**Fig. 6.** Helical wheel projection of human B2 receptor C-terminal residues 310 through 321. The B2 receptor residues are numbered according to Hess et al. (1992) (outside parenthesis). The corresponding residues in bovine rhodopsin are indicated for reference (inside parenthesis). The length of the projection is based on that of helix 8 identified in the crystal structure of bovine rhodopsin (Palczewski et al., 2000). Indicated also are the charged face and the hydrophobic face of this putative B2 receptor helix to emphasize its amphipathic character.

rupted in the B1 receptor by the replacement of $Arg^{311}$ with $Leu^{318}$, the only nonconserved substitution in this region. This may be the reason for the apparent independence of the B1 receptor on the C-terminal domain for basic G protein coupling.

That $Arg^{311}$, the second residue in the putative eighth B2 receptor helix and the BBXB motif, is somehow involved in $G_{q/11}$ coupling is evident from the dramatic functional effect of Ala mutation of this residue in the intact B2 receptor. Contrary to what one might expect, an approximately 5-fold increase in receptor coupling efficacy was observed by this mutation. Ala mutation of $Lys^{314}$, on the other hand, which is positioned one residue beyond the above motif but remaining as part of the putative helix, only had a small effect on $G_{q/11}$ coupling. However, $Lys^{314}$ is probably readily replaceable by Ala in terms of helix propensity. Furthermore, Ala mutation of the downstream $Cys^{324}$ and $Cys^{329}$, which are palmitoylated (Pizard et al., 2001), had no significant effect on coupling. Nevertheless, these results suggest that $Arg^{311}$ exerts an inhibitory effect on $G_{q/11}$ coupling. As shown in Fig. 6, $Arg^{311}$ is located on the charged face of the eighth helix. The significance of this orientation is not known because the precise role of this helix in G protein coupling has not been established. Substituting Arg in the corresponding position in the B1 receptor, which is occupied by $Leu^{318}$ and represents the only nonconserved position among the first eight C-terminal residues in the two receptors, provides further evidence for the inhibitory effect of this residue and its contribution to the different coupling efficacies of the receptors.

The mechanism of $Arg^{311}$ inhibition is not known, although it requires the basic charge of the residue. Because Ala mutation of $Arg^{311}$ was not effective in either B2Stop313 or B2Stop321, $Arg^{311}$ inhibition in the B2 receptor seems to be dependent on additional residues downstream of $Tyr^{320}$. That it can inhibit on its own under some conditions was suggested by the fact that its inhibitory effect in the B1 receptor was independent of the length of the C-terminal domain. The inhibitory action of $Arg^{311}$ in the B2 receptor may be related to that of a cluster of four basic residues in the membrane-proximal portion of the C-terminal domain of the metabotropic glutamate receptor 1, which also couples through $G_{q/11}$ to phospholipase C$\beta$ (Mary et al., 1998). The inhibitory action of this cluster becomes apparent in the shorter splice variants of this receptor, but not in the longer ones, by the loss of both ligand-independent and agonist-dependent PI hydrolysis. It was proposed that because this cluster is common to all metabotropic glutamate receptor 1 variants, the additional C-terminal residues of the long splice variants suppress the inhibitory effect of the cluster. This is different from the B2 receptor in which $Arg^{311}$ mutation was effective in the full-length receptor.

The ligand-independent and the agonist-dependent B1 receptor activities were equally affected by C-terminal truncation, suggesting that it is the responsiveness of the spontaneously formed R* rather than the interconvertion of R and R* that is changed by this modification. Equal effects on these activities were also observed when four of the five residues in the Ser/Thr cluster were mutated in the WT B2 receptor (Fathy et al., 1999) and B1(B2ICIV) (Leeb-Lundberg et al., 2001). This result may be distinguished from that obtained by mutating $Asn^{121}$ in the third transmembrane domain of the B1 receptor, which caused an increase primarily in the ligand-independent activity (Leeb-Lundberg et al., 2001). Together, these results define two putative mechanisms of controlling ligand-independent activity in the B1 receptor. One mechanism controls the amount of R* by influencing the receptor isomerization constant, and another mechanism regulates the coupling efficacy of the spontaneously formed R*. Nevertheless, a few mutations presented in this report influenced $I_A$ and $I_B$ selectively. One of these mutations involved B2 receptor $Arg^{311}$, which upon conversion to Ala in the B2 receptor to make $B2A^{311}$ led to a selective increase in $I_A$ and upon substitution at the corresponding position in the B1 receptor to make $B1R^{318}$ led to a selective decrease in $I_A$. A selective increase in $I_A$ was also observed upon substituting the B1 receptor C-terminal domain in the B2 receptor, whereas a selective increase in $I_B$ was observed upon mutating $Ser^{346}$ to Ala in B1 ($B2ICIVA^{Ser/Thr}$) to make $B1(B2ICIVA^{Ser/Thr})A^{346}$. These results argue that the spontaneously formed activated state and the agonist-promoted activated state are not identical and that the two are differently regulated.

Whereas a relatively high coupling efficacy may be expected of the B1 receptor considering its inducible character, to our knowledge, no study has directly evaluated and compared the coupling efficacies of the B1 and B2 receptors in vivo. This is presumably due to the difficulty in accurately determining the relatively low level of expression of the receptor under normal conditions. Naïve cultured vascular smooth muscle cells from the rabbit superior mesenteric artery express B1 and B2 receptors (Tropea et al., 1993) at a ratio of approximately 1:3 (D. S. Kang and L. M. F. Leeb-Lundberg, unpublished observations). On the other hand, the maximal B1 receptor-promoted PI response in these cells is at least as high as that of the B2 receptor response (Tropea et al., 1993). Although these results suggest that the B1 receptor is more effectively coupled to the PI response than the B2 receptor also in the native environment of these receptors in the same cell, this issue certainly has to be investigated in more detail.

In conclusion, we have shown that the C-terminal domains of the B1 and B2 receptors both participate in $G_{q/11}$ coupling but do so in different ways. The B2 receptor is critically dependent on this domain for coupling, possibly due to the formation of an eighth receptor helix by this domain. This may render the receptor sensitive to several negative regulatory epitopes in the domain, including $Arg^{311}$, as well as the previously identified serines and threonines in the Ser/Thr cluster. In contrast, the B1 receptor is not directly dependent on the C-terminal domain for coupling. However, this domain contains positive regulatory epitopes in addition to lacking the negative regulatory epitopes present in the B2 receptor. We propose that these structural features together provide the B1 and B2 receptors with their drastically different efficacies of $G_{q/11}$ coupling.





**References**

Austin CE, Faussner A, Robinson HE, Chakravarty S, Kyle DJ, Bathon JM, and Proud D (1997) Stable expression of the human kinin B1 receptor in chinese hamster ovary cells: characterization of ligand binding and effector pathways. *J Biol Chem* **272:**11420–11425.

Bhoola KD, Figueroa CD, and Worthy K (1992) Bioregulation of kinins: kallikreins, kininogens and kininases. *Pharmacol Rev* **44:**1–80.

Blaukat A, Herzer K, Schroeder C, Bachmann M, Nash N, and Muller-Esterl W (1999) Overexpression and functional characterization of kinin receptors reveal subtype-specific phosphorylation. *Biochemistry* **38:**1300–1309.

Blaukat A, Pizard A, Breit A, Wernstedt C, Alhenc-Gelas F, Müller-Esterl W, and

Dikic I (2001) Determination of bradykinin B2 receptor in vivo phosphorylation sites and their role in receptor function. *J Biol Chem* **276:**40431–40440.
Chakrabartty A and Baldwin RL (1995) Stability of alpha-helices. *Adv Protein Chem* **46:**141–176.
Claeysen S, Sebben M, Becamel C, Bockaert J, and Dumuis A (1999) Novel brain-specific 5-$HT_4$ receptor splice variants show marked constitutive activity: role of the C-terminal intracellular domain. *Mol Pharmacol* **55:**910–920.
Couture R, Harrisson M, Vianna RM, and Cloutier F (2001) Kinin receptors in pain and inflammation. *Eur J Pharmacol* **429:**161–176.
Fathy DB, Leeb T, Mathis SA, and Leeb-Lundberg LMF (1999) Spontaneous human B2 bradykinin receptor activity determines the action of partial agonists as agonists or inverse agonists: effect of basal desensitization. *J Biol Chem* **274:**29603–29606.
Gether U (2000) Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. *Endocr Rev* **21:**90–113.
Gutowski S, Smrcka A, Nowak L, Wu DG, Simon M, and Sternweis PC (1991) Antibodies to the alpha q subfamily of guanine nucleotide-binding regulatory protein alpha subunits attenuate activation of phosphatidylinositol 4,5-bisphosphate hydrolysis by hormones. *J Biol Chem* **266:**20519–20524.
Hecquet C, Tan F, Marcic BM, and Erdos EG (2000) Human bradykinin B2 receptor is activated by kallikrein and other serine proteases. *Mol Pharmacol* **58:**828–836.
Hess JF, Borkowski JA, Young GS, Strader CD, and Ransom RW (1992) Cloning and pharmacological characterization of a human bradykinin (BK-2) receptor. *Biochem Biophys Res Commun* **184:**260–268.
Hogger P, Shockley MS, Lameh J, and Sadee W (1995) Activating and inactivating mutations in N- and C-terminal i3 loop junctions of muscarinic acetylcholine Hm1 receptors. *J Biol Chem* **270:**7405–7410.
Jones S, Brown DA, Milligan G, Willer E, Buckley NJ, and Caulfield MP (1995) Bradykinin excites rat sympathetic neurons by inhibition of M current through a mechanism involving B2 receptors and G alpha q/11. *Neuron* **14:**399–405.
Krupnick JG and Benovic JL (1998) The role of receptor kinases and arrestins in G protein-coupled receptor regulation. *Annu Rev Pharmacol Toxicol* **38:**289–319.
Lamb ME, de Weerd WFC, and Leeb-Lundberg LMF (2001) Agonist-promoted trafficking of human bradykinin receptors: arrestin- and dynamin-independent sequestration of the B2 receptor in HEK293 cells. *Biochem J* **355:**741–750.
Leeb-Lundberg LMF, Kang DS, Lamb ME, Mathis S, and Fathy DB (2001) The human B1 bradykinin receptor exhibits high ligand-independent, constitutive activity: roles of the third transmembrane and C-terminal domains. *J Biol Chem* **276:**8785–8792.
Lee NH, Geoghagen NS, Cheng E, Cline RT, and Fraser CM (1996) Alanine scanning mutagenesis of conserved arginine/lysine-arginine/lysine-X-X-arginine/lysine G protein-activating motifs on m1 muscarinic acetylcholine receptors. *Mol Pharmacol* **50:**140–148.
Marceau F, Hess JF, and Bachvarov DR (1998) The B1 receptors for kinins. *Pharmacol Rev* **50:**357–386.
Marie J, Richard E, Pruneau D, Paquet J-L, Siatka C, Larguier R, Ponce C, Vassault P, Groblewski T, Maigret B, et al. (2001) Control of conformational equilibria in the human B2 bradykinin receptor: modeling of nonpeptidic ligand action and comparison to the rhodopsin structure. *J Biol Chem* **276:**41100–41111.
Mary S, Gomeza J, Prezeau L, Bockaert J, and Pin J-P (1998) A cluster of basic residues in the carboxy-terminal tail of the short metabotropic glutamate receptor 1 variants impairs their coupling to phospholipase C. *J Biol Chem* **273:**425–432.
Mathis SA, Criscimagna NL, and Leeb-Lundberg LMF (1996) B1 and B2 kinin receptors mediate distinct patterns of intracellular $Ca^{2+}$ signaling in single cultured vascular smooth muscle cells. *Mol Pharmacol* **50:**128–139.
Menke JG, Borkowski JA, Bierilo KK, MacNeil T, Derrick AW, Schneck KA, Ransom RW, Strader CD, Linemeyer DL, and Hess JF (1994) Expression cloning of a human B1 bradykinin receptor. *J Biol Chem* **269:**21583–21586.
Offermanns S, Zhao L-P, Gohla A, Sarosi I, Simon MI, and Wilkie TM (1998) Embryonic cardiomyocyte hypoplasia and craniofacial defects in $G\alpha_q/G\alpha_{11}$-mutant mice. *EMBO (Eur Mol Biol Organ) J* **17:**4304–4312.
Okamoto T and Nishimoto I (1992) Detection of G protein-activator regions in M4 subtype muscarinic, cholinergic and alpha 2-adrenergic receptors based upon characteristics in primary structure. *J Biol Chem* **267:**8342–8346.
Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, et al. (2000) Crystal structure of rhodopsin: a G protein-coupled receptor. *Science (Wash DC)* **289:**739–745.
Phagoo SB, Poole S, and Leeb-Lundberg LMF (1999) Autoregulation of bradykinin receptors: agonists in the presence of interleukin-1$\beta$ shift the repertoire of receptor subtypes from B2 to B1 in human lung fibroblasts. *Mol Pharmacol* **56:**325–333.
Pitcher JA, Freedman NJ, and Lefkowitz RJ (1998) G protein-coupled receptor kinases. *Annu Rev Biochem* **67:**653–692.
Pizard A, Blaukat A, Michineau S, Dikic I, Muller-Esterl W, Ahlenc-Gelas F, and Rajerison RM (2001) Palmitoylation of the human bradykinin B2 receptor influences ligand efficacy. *Biochemistry* **40:**15743–15751.
Pizard A, Blaukat A, Muller-Esterl W, Ahlenc-Gelas F, and Rajerison RM (1999) Bradykinin-induced internalization of the human B2 receptor requires phosphorylation of three serine and two threonine residues at its carboxyl tail. *J Biol Chem* **274:**12738–12747.
Prado G, Taylor L, and Polgar P (1997) Effects of intracellular tyrosine residue mutation and carboxyl terminus truncation on signal transduction and internalization of the rat bradykinin B2 receptor. *J Biol Chem* **272:**14638–14642.
Regoli D and Barabe J (1980) Pharmacology of bradykinin and related kinins. *Pharmacol Rev* **32:**1–46.
Samama P, Cotecchia S, Costa T, and Lefkowitz RJ (1993) A mutation-induced activated state of the beta 2-adrenergic receptor. Extending the ternary complex model. *J Biol Chem* **268:**4625–4636.
Sano T, Ohyama K, Yamano Y, Nakagomi Y, Nakazawa S, Kikyo M, Shirai H, Blank JS, Exton JH, and Inagami T (1997) A domain for G protein coupling in carboxyl-terminal tail of rat angiotensin II receptor type 1A. *J Biol Chem* **272:**23631–23636.
Schanstra JP, Bataille E, Marin Castano ME, Barascud Y, Hirtz C, Pesquero JB, Pecher C, Gauthier F, Girolami JP, and Bascands JL (1998) The B1-agonist [des-$Arg^{10}$]-kallidin activates transcription factor NF-$\kappa$B and induces homologous upregulation of the bradykinin B1-receptor in cultured human lung fibroblasts. *J Clin Invest* **101:**2080–2091.
Scheer A, Fanelli F, Costa T, De Benedetti PG, and Cotecchia S (1996) Constitutively active mutants of the alpha 1B-adrenergic receptor: role of highly conserved polar amino acids in receptor activation. *EMBO (Eur Mol Biol Organ) J* **15:**3566–3578.
Tropea MM, Gummelt D, Herzig MCS, and Leeb-Lundberg LMF (1993) B1 and B2 kinin receptors on rabbit superior mesenteric artery smooth muscle cells: receptor-specific stimulation of inositol phosphate formation and arachidonic acid release by des-$Arg^9$-bradykinin and bradykinin. *J Pharmacol Exp Ther* **264:**930–937.
Wess J (1997) G-protein-coupled receptors: molecular mechanisms involved in receptor activation and selectivity of G-protein recognition. *FASEB J* **11:**346–354.
Wess J (1998) Molecular basis of receptor/G-protein-coupling selectivity. *Pharmacol Ther* **80:**231–264.

**Address correspondence to:** L. M. Fredrik Leeb-Lundberg, Ph.D., Department of Biochemistry, The University of Texas Health Science Center at San Antonio, Mail Code 7760, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900. E-mail: lundberg@biochem.uthscsa.edu

MOL PHARM

# Correction to "Negative and positive regulatory epitopes in the C-terminal domains of the human B1 and B2 bradykinin receptor subtypes determine receptor coupling efficacy to $G_{9/11}$-mediated phospholipase C$\beta$ activity "

In the above article [Dong Soo Kang DS and Leeb-Lundberg LMF (2002) *Mol Pharmacol* **62:**281–288], the title was incorrect. The correct title is "Negative and positive regulatory epitopes in the C-terminal domains of the human B1 and B2 bradykinin receptor subtypes determine receptor coupling efficacy to $G_{q/11}$-mediated phospholipase C$\beta$ activity".

We apologize for this error and regret any confusion or inconvenience it may have caused.



MOLECULAR PHARMACOLOGY

aspet