Influence of the Cytosolic Carboxyl Termini of Human B1 and B2 Kinin Receptors on Receptor Sequestration, Ligand Internalization, and Signal Transduction*

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To determine the role of the cytoplasmic carboxyl termini of human B1 and B2 kinin receptors (B1KR and B2KR, respectively) in the internalization of their respective ligands, des-Arg¹⁰-kallidin and bradykinin (BK), both wild type receptors, as well as truncated B2KRs, a mutated B2KR, and chimeric receptors were stably expressed in Chinese hamster ovary cells. Incubation of [³H]BK at 37 °C with cells expressing wild type B2KR resulted in pronounced and rapid ligand internalization (~80% after 10 min). By contrast, incubation of [³H]-labeled des-Arg¹⁰-kallidin with cells expressing B1KR resulted in a modest, slow internalization of the ligand (~20% after 10 min). Replacement, from Cys³²⁴, of the cytoplasmic carboxyl terminus of the B2KR with that of the B1KR from Cys³³⁰ (both Cys residues are putative palmitoylation sites) greatly reduced ligand internalization (~40% after 10 min) without significantly altering Kᵢ or ligand-induced signal activation. By marked contrast, the corresponding replacement, of the sequence from Cys³³⁰ of the cytoplasmic carboxyl terminus of the B1KR with the segment of the B2KR, led to a striking increase of ligand internalization (~75% within 10 min) without altering Kᵢ or ligand-induced signal activation. Truncation of the B2KR to within three amino acids of Cys³²⁴ (truncation at Gly³²⁷) led to strongly reduced ligand internalization (~40% after 10 min). Truncation of the B2KR up to Lys³⁵ almost completely abolished internalization of [³H]BK (10% after 10 min). This additional reduction is apparently not caused by the loss of the potential palmitoylation site at Cys³²⁴ since a B2KR with a point mutation of Cys³²⁴ to Ala internalized [³H]BK as rapidly as the wild type B2KR.

From these results we conclude that the cytoplasmic carboxyl terminus of the human B2KR contains sequences that are necessary and sufficient to permit rapid ligand-induced sequestration of human kinin receptors and internalization of their agonists.

*Kinins are small peptides that are released from high molecular weight precursors, called kininogens, by limited proteolysis performed by enzymes known as kallikreins. They exert their large array of biologic effects at the cellular level via two types of receptors, B1 and B2 kinin receptors (B1KR and B2KR, respectively) (1–5). The B2KR is constitutively expressed in a variety of tissues and cell lines, binding with high affinity to bradykinin (BK) and to Lys-BK (Kallidin), but not to their respective carboxypeptidase degradation products des-Arg¹⁰-BK and des-Arg¹⁰-kallidin (DAK). The B1KR, in contrast, is expressed de novo under certain pathological conditions and exhibits no affinity for BK but a high affinity for DAK and a low affinity for kallidin and des-Arg²⁵-BK (7). Both human kinin receptors have been cloned recently (8, 9). Hydrobodically analysis of their amino acid sequence revealed that they both have seven hydrophobic domains that are typical for members of the superfamily of G-protein-coupled receptors (GPCRs). The amino acid sequences of both receptors show an overall homology of only 36%, however, with higher areas of homology in the transmembrane regions (9). Despite their relatively low homology, the receptors appear to share similar signal transduction pathways, such as activation of phospholipase C that leads to the release of inositol phosphates and an increase in intracellular [Ca²⁺] levels, in response to binding of agonists (9). While it is already well established that B2KR couples to phospholipase C via the G-protein Gₛ (10, 11), we demonstrated only recently that binding of DAK to B1KR leads to the activation of Gₛ and Gₛ₁₂ (7). The kinin receptors appear to differ, however, in mechanisms of signal “termination.” Several studies showed that B2KR responds to agonist binding with ligand-induced receptor sequestration (12, 13) and ligand internalization (14, 15). These properties of the B2KR may be responsible, at least in part, for the rapid decrease in responsiveness of the involved cells to a second stimulation with BK (desensitization). The B1KR, in contrast, internalizes its ligand very slowly and shows almost no short term desensitization (7).

For GPCRs with very long third intracellular loops, such as the muscarinic receptor (almost 300 amino acids), important sequences for ligand-induced receptor sequestration and ligand internalization have been identified in this third loop (16, 17). In GPCRs with short third intracellular loops, other domains may contribute to the signal for ligand internalization. For receptors including the β-adrenergic, angiotensin II, gastrin-releasing peptide, parathyroid hormone receptors, and ß-opioid receptor, domains have been localized to the cytoplasmic tail, particularly to sequences enriched in serine and threonine residues (18–23). Both kinin receptor subtypes have short third intracellular loops, and their cytoplasmic COOH termini
share no homology after the putatively palmitoylated cysteine. Therefore, we asked whether their opposite patterns of internalization are the consequence of either a positive sequence (in the case of the B2KR) or an inhibitory sequence (B1KR) for their cytoplasmic tail.

The results of our study demonstrate for the first time (i) that the information encoded in the cytoplasmic tail of the B2KR is necessary and sufficient for ligand-induced sequestration of B2 and also B1 kinin receptors; (ii) that loss of a putative palmitoylation site in the cytoplasmic tail of B2KR does not interfere with ligand internalization or receptor sequestration; (iii) that the B1KR, in contrast to B2KR, does not respond to binding of an agonist with receptor sequestration; and (iv) that exchange of the cytoplasmic tail does not transfer the desensitization pattern.

EXPERIMENTAL PROCEDURES

Materials— Stock cultures of IMR 90 lung fibroblasts were grown in Dulbecco’s modified Eagle’s medium with high glucose, 2 mM glutamine, 10% fetal calf serum, and penicillin/streptomycin. Monolayers were maintained with limiting dilution and screened for binding of the appropriate ligand. All stock cultures were kept under constant selection pressure of G418 (800 μg/ml) whereas cells seeded in dishes/wells were maintained without G418 and used within 2–5 days.

Construction of pcDNA3-B2, B2KR Truncations, and the B2KR Mutation—Construction of the pcDNA3-B2 vector containing the B2 receptor (pcDNA3-B1), was kindly provided by Dr. J. Fred Hess (Merck).

Cell Culture—Stock cultures of IMR 90 lung fibroblasts were grown in Dulbecco’s modified Eagle’s medium with high glucose, 2 mM glutamine, 10% fetal calf serum, and penicillin/streptomycin. Most additives, Opti-MEM, Geneticin (G418 sulfate), and fetal calf serum were bought from Life Technologies, Inc. Penicillin/streptomycin, culture media, most additives, Opti-MEM, Geneticin (G418 sulfate), and fetal calf serum were bought from Life Technologies, Inc. Penicillin/streptomycin was obtained from Biofluids Inc. CaptoPril was purchased from Sigma. All other reagents were of analytical grade and commercially available. A pcDNA3 vector, harboring the cDNA encoding the B1 receptor (pcDNA3-B1), was constructed by us using the T7 polymerase from Stratagene. All primers were synthesized by Life Technologies, Inc.

Construction of pcDNA3-B2, B2KR Truncations, and the B2KR Mutation—pcDNA3-B2—mRNA from human synovial fibroblasts was used as starting material to obtain the cDNA encoding the B2 receptor (pcDNA3-B1), by reverse transcriptase polymerase chain reaction (PCR). The PCR product was then cloned between the BamHI and Xhol sites of the pcDNA3 vector (Invitrogen). The sequence of the insert was determined by automated sequencing using the dideoxynucleotide chain termination method. The plasmid was isolated by limiting dilution and screened for binding of the appropriate ligand. pcDNA3-B2 was used as template to obtain the truncated mutants Gly327 and Lys315 and the point mutation C324A using standard PCR methodology with appropriate oligonucleotides.

Construction of B1/B2 Receptor Chimera—For the synthesis of the chimera, in which the cytoplasmic tail of the B1KR was replaced with that of the B2KR (B2CB1), a first PCR was performed with the pcDNA3-B1 vector as template using a T7 primer and a chimeric antisense primer 5′-CCTGCTACCCCCCTTCTGCCATTGTATTAAAGT-TCC-3′ corresponding to the B1 sequence, starting at Cys324 (encoded by TGC) and was identical with the published human B2KR sequence (8). This pcDNA3 vector harboring the B2KR (pcDNA3-B2) was subsequently used as template to obtain the truncated mutants Gly327 and Lys315 and the point mutation C324A using standard PCR methodology with appropriate oligonucleotides.

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The antisense primer Sp6. The products of both reactions, by hybridization with appropriate oligonucleotides.

By reverse transcriptase polymerase chain reaction (PCR). The PCR product, when cloned between the sites of the pcDNA3-B2 vector, was used as template for the first reaction with pcDNA3-B2 as template and 5′-GATGTCGACCACCCTAATACTGCCTCGGC-3′ as sense primer in the second PCR with pcDNA3-B1 as template. The accuracy of the sequence of the chimeras was confirmed by sequencing selected clones using the dideoxynucleotide method (24). Plasmids were obtained using JM109 bacteria (Promega) and a plasmid purification midikit from Qiagen.

Transfection and Selection—20 μg of plasmid DNA in 300 μl of Opti-MEM and 30 μl of Lipofectamine (Life Technologies, Inc.) in 270 μl of Opti-MEM were combined and kept at room temperature for 15 min to obtain a precipitate. After the addition of 4.6 ml of Opti-MEM, the whole mixture was added to subconfluent (40–70%) CHO cells in a 80-cm² flask that had been rinsed with 5 ml of Opti-MEM and incubated with another 5 ml for 15 min at 37 °C. After 16–36 h, the medium was replaced with complete medium containing G418 (800 μg of geneticin/ml) to start the selection of stably transfected cells. Medium was changed every 4 days, and after about 15 days individual clones were isolated by limiting dilution and screened for binding of the appropriate ligand. All stock cultures were kept under constant selection pressure of G418 (800 μg/ml) whereas cells seeded in dishes/wells were maintained without G418 and used within 2–5 days.

H-Ligand Binding Studies—For the determination of dissociation constants (Kᵢ) and receptor numbers (Bmax), cell monolayers in 24- or 12-well (CHO) or 6-well trays (IMR 90) were washed three times with wash buffer (40 mM PIPES, 109 mM NaCl, 5 mM KCl, 0.1% glucose, 0.05% bovine serum albumin, 2 mM CaCl₂, 1 mM MgCl₂, pH 7.4) followed by preincubation at 37 °C with incubation buffer (wash buffer supplemented with 0.2% phosphatidylcholine and 100 μM captopril). The tryps were subsequently placed on ice, and the indicated concentrations of [³H]BK or [³H]DAK were added for determination of total B2KR and B1KR binding, respectively. Nonspecific binding was determined in the presence of 5 μM of the appropriate unlabeled ligand. After an incubation time of at least 90 min, the cell monolayers were washed four times with ice-cold incubation buffer, lysed with 0.3% sodium hydroxide, and transferred quantitatively into scintillation vials. Radioactivity of the samples was measured in a β-counter after the addition of a 10-fold volume of scintillation fluid. Specific binding was calculated as the difference between total binding and nonspecific binding.

H-Ligand Internalization—To determine the internalization of receptor-bound ligand, cell monolayers were washed three times with prewarmed (37 °C) wash buffer and preincubated at 37 °C with incubation buffer for at least 30 min. Internalization was started by replacing the buffer with incubation buffer containing the indicated concentrations of [³H]ligand with or without 5 μM unlabeled ligand. After the indicated time of incubation, the medium was changed with 10 ml of complete medium containing G 418 (800 μg/ml) and specific B2KR or B1KR binding was determined at 0 °C as described above using approximately 2 nM [³H]BK or 1 nM [³H]DAK. After the indicated times, the cells were washed four times with 0.5 ml of ice-cold wash buffer and treated on ice for 10 min with dissociation solution to remove all unbound surface-bound ligand. The monolayers were washed again four times with ice-cold wash buffer, and specific B2KR or B1KR binding was determined at 0 °C as described above using approximately 2 nM [³H]BK or 1 nM [³H]DAK.

Stimulation of Total Inositol Phosphate Release—80% confluent cell monolayers of stably transfected clones grown in 12-well trays were labeled for 24–48 h with 2 μCi of myo-[³H]inositol in 0.5 ml of α-minimum essential medium supplemented with 0.05% bovine serum albumin and penicillin/streptomycin. Monolayers were rinsed with wash buffer, equilibrated in prewarmed incubation buffer containing 50 mM LiCl for 15–30 min, and stimulated with the appropriate agonist at 37 °C. The stimulation was terminated by exchanging the buffer for 0.75 ml of an ice-cold solution of 20 mM formic acid and by placing the trays on ice for 30 min. After transferring the supernatants quantitatively to another 0.75 ml of ice-cold solution of 20 mM ammonium hydroxide solution was added, and the mixtures were applied to AG 1-X8 anion exchange columns (1-ml volume). The columns were washed with 1 ml of 1.8% ammonium hydroxide, followed by 9 ml of 60 mM sodium formate, 5 mM tetraborate buffer, and total inositol phosphates were eluted with 1.5 ml of 4 M ammonium formate, 0.2 M formic acid.

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FIG. 1. Comparison of the internalization of [3H]BK or [3H]DAK in lung fibroblasts IMR 90 and transfected CHO cells. Six-well trays of IMR 90 cells (○, △) or 12-well trays of CHO cells transfected with either the B2 (○, WT B2) or the B1 (△, WT B1) receptor gene were incubated with approximately 2 nM [3H]BK (●, ○) or 1 nM [3H]DAK (▲, △), respectively. At the indicated times, ligand internalization was determined as described under “Experimental Procedures.”

For the determination of the EC50 values, the cell monolayers were stimulated for 30 min with increasing concentrations of agonist. For the time curves, stimulation was carried out with 1 μM of the appropriate agonist.

Statistics—All points in the figures represent the mean ± S.E. of at least three experiments done in triplicate. For internalization and sequestration experiments with transfected CHO cells, G418-selected, but not cloned, cell populations and at least one stably transfected clone were evaluated. Data were analyzed using paired or unpaired t tests, as appropriate. Significance was assumed for values of p < 0.05.

RESULTS

Ligand Internalization of Endogenously and Heterologously Expressed Kinin Receptors—Human embryonic lung fibroblasts IMR 90 represent one of the few human cell lines that naturally express both types of kinin receptors, but B1KR expression is low (5,000 sites/cell) compared with that of B2KR (70,000 sites/cell) (9). To date it is not known whether the B1KR becomes sequestered upon ligand stimulation as has been described for the human B2KR (12, 13), and it was only very recently that we reported the almost complete lack of internalization of DAK after binding to a B1KR expressed in CHO cells (7). Since this lack of internalization might have been due to the expression of the B1KR in a noncompatible cell system, we used the acetic acid treatment method (25) that has been used to study the internalization of a variety of hormones to examine the behavior of the B1KR in IMR 90 cells. An added benefit to the study of IMR 90 cells is the ability to compare internalization/sequestration of both kinin receptor types in the same cell line. The B2KR of IMR 90 cells internalized [3H]BK very quickly reaching a plateau of 75% internalization of bound ligand after 10 min (Fig. 1). This is similar to what has been described for the B2KR in other cell lines (14, 15). In contrast, even after 30 min of incubation, less then 20% of bound [3H]DAK was found in an acid-resistant compartment (Fig. 1). We considered that the low level of B1KR expression in IMR 90 cells might be responsible for slow [3H]DAK internalization. We addressed this possibility using two approaches. First, we enhanced expression of B1KR in IMR 90 cells by preincubation with IL-1 (1 ng/ml) for 24 h as previously reported (not shown) (9). Second, we expressed high numbers of each receptor type in CHO cells, resulting in up to 67 fmol of B1KR/106 cells and up to 94 fmol of B2KR/106 cells. In each case, internalization rates for the respective ligands for each receptor were nearly identical to those observed in untreated IMR 90 cells (Fig. 1 and Ref. 7). These studies confirmed that the markedly different internalization rates of the B1KR and B2KR ligands are a property of the receptors themselves rather than simply a reflection of variable receptor density. Nontransfected CHO cells showed no uptake (internalization) of labeled ligand in these conditions (not shown).

For all described experiments, CHO cells were transfected with pcDNA3 vectors, harboring inserts encoding the B1KR or the B2KR. Geneticin-selected, uncloned populations of both wild type receptors (WT B1 and WT B2) were used to isolate up to 20 individual clones. The expression levels of these G418-resistant clones were quite variable, ranging from no expression of kinin receptors at all to almost 500 fmol/106 cells. Nonetheless, for all wild type and mutant kinin receptors expressed in CHO cells, the pattern of ligand internalization was quite consistent between selected, uncloned populations and/or clones with varying expression levels (data not shown), similar to what has been reported for the angiotensin II receptor (20).

[3H]-Ligand Internalization of Receptor Chimeras—In view of the role of the cytoplasmic tail in conveying a signal for internalization in a number of nonkinin receptors (18–23, 26), we next asked (i) if the domains responsible for the rapid internalization of BK by the B2KR and the slow internalization of DAK by the B1KR were contained in their respective cytoplasmic tails and (ii) if so, whether exchange of the tails between the two kinin receptor types would alter their respective ligand internalization rates. To address these questions, we created chimeric receptors in which the cytoplasmic tails were swapped at a highly conserved (putative) palmitoylation site (Cys324 in B2KR and Cys330 in B1KR) (Fig. 2).

This exchange did not significantly change the affinities of the receptors for their respective ligands at 0 °C (Table 1). There was, however, a dramatic change in their internalization behavior at 37 °C. As can be seen in Fig. 2, the B2 chimera with the B1 tail (B2CB1) internalized [3H]BK at a considerably lower rate than WT B2, whereas the B1 chimera with the B2 tail (B1CB2) now internalized [3H]DAK almost as quickly as
WT B2 internalized its ligand [3H]BK. 

Ligand Internalization of Truncated B2KR—To examine whether the low internalization rate of the B2CB1 chimera was due to an inhibitory sequence in the cytoplasmic tail of B1KR, we examined the effect of truncation of the B2KR on its rate of ligand internalization. Cy324 was felt to be the ideal site for truncation, since this would maintain consistency with the site of cytoplasmic tail exchange in the previous experiments. Cy324 is presumed to be palmitoylated, however, and several fatty acid transferases require additional amino acids adjacent to the target amino acid (Cys) to function properly. Therefore, we performed a more conservative truncation at Gly327. This truncation resulted in a significant reduction in [3H]BK internalization to a rate comparable with that of the B2CB1 chimera (Fig. 3). Because internalization was still considerably faster in the B2KR (Fig. 4, bottom), however, sequestration of the truncated receptor Lys315 was observed. Gly327 receptors was markedly reduced, while almost no sequestration of the chimeric B2CB1 and truncation resulted in a significant reduction in [3H]BK internalization. Cys324 was felt to be the ideal site for truncation, since this would maintain consistency with the site of cytoplasmic tail exchange in the previous experiments.

### Table I

| Cell line       | $K_d$ (nM) | $B_{max}$ (fmol/10^6 cells) | EC_{50} ± S.E. |
|-----------------|------------|-----------------------------|---------------|
| WT B2           | 1.3        | 94                          | 0.32 ± 0.21 (n=4) |
| WT B1           | 0.3a       | 67                          | 0.42 ± 0.20 (n=4) |
| B2CB1           | 2.6        | 472                         | 0.15 ± 0.08 (n=4) |
| B1CB2           | 0.4        | 145                         | 0.65 ± 0.23 (n=5) |
| B2/Gly327stop   | 0.9        | 65                          | 0.77 ± 0.22 (n=3) |
| B2/Lys315stop   | 1.4        | 118                         | 2.24 ± 0.25 (n=5) |
| B2/C324A        | 1.1        | 42                          | ND            |

*a Data from Austin et al. (7).

*b $p < 0.001$ versus WT B2.

*c Not determined.

The GPCRs are a superfamily of receptors that transmit extracellular signals to cytosolic and membrane-associated effector molecules via coupling to one or more heterotrimeric G-proteins (27, 28). In the case of kinins, ligand activation of...
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The putative palmitoylation site at Cys 324 is not involved, and some additional requirement for receptor internalization appears to be located in the sequence between Lys315 and Gly327. The putative palmitoylation site at Cys324 is not involved, because the mutant C324A internalizes and sequesters very similarly to the WT B2 (Fig. 3 and Fig. 4, top).

The low but distinct internalization rate of [3H]DAK in WT B1 or IMR 90 fibroblasts may best be explained by a slow continuous turnover of B1KRs that, in a ligand-independent manner, transports bound ligand inside the cell, since there is no significant reduction of surface binding even after incubation with 1 μM DAK for 30 min (Fig. 4, bottom). In other words, DAK has no direct influence on the number of B1 surface receptors. In contrast, after the exchange of the cytoplasmic tail of the B1KR with that of the B2KR, rapid internalization of [3H]DAK was observed (Fig. 3), and DAK exposure resulted in a reduction of the number of B1KRs on the cell surface (Fig. 4, bottom). This clearly indicates that the cytoplasmic tail of the B2 receptor, on the one hand, provides the B1KR with the ability to undergo internalization but, on the other hand, now is under the control of the remaining domains of B1KR (i.e. induction of sequestration is dependent on DAK, and the receptor has not simply achieved a higher rate of constitutive recycling). Thus, despite the low homology of only 36% between both receptors (9), nearly complete transfer of the internalization pattern of B2KR is possible via transfer of its cytoplasmic tail.

It is important to understand what sequence(s) in the cytoplasmic tail of B2KR provides signals for sequestration and how it becomes accessible for the cellular machinery mediating receptor sequestration. Several groups have shown the importance of serine and threonine residues for receptor internalization in the cytoplasmic tails of GPCRs (18–23). A good possibility for explaining the marked reduction in ligand internalization exhibited by the Gly327 truncation, therefore, is the removal of the sequence Thr-Ser-Ile-Ser in the cytoplasmic tail, a sequence that is similar to the sequence Ser-Thr-Leu in the angiotensin AT1 receptor that was determined to play an essential role in its internalization (19). Phosphorylation of serine and threonine residues of other GPCRs by receptor kinases has been shown to lead to coupling of β-arrestins, which are involved in receptor internalization (32). A similar mechanism might apply for the internalization of the B2KR, since transient phosphorylation of serine and threonine residues of the
B2KR within minutes after stimulation with BK has been reported (33). This phosphorylation took place at least in part, if not exclusively, in the cytoplasmic tail of the B2KR. Partial inhibition of B2KR internalization by concanavalin A led to reduced dephosphorylation of the B2KR, suggesting that a role for receptor internalization is to facilitate receptor dephosphorylation, as similarly proposed for the β-adrenergic receptor (18, 33). A study with β-adrenergic receptors demonstrated that receptors that were resistant to phosphorylation, either because of a truncation proximal to the palmitoylation site or because of point mutations of all critical phosphorylation sites (i.e. serines and threonines), displayed markedly reduced receptor sequestration (32). A phosphorylated cytoplasmic tail, however, may facilitate receptor internalization through increased interaction with β-arrestins but cannot be the only contact site in GPCRs for β-arrestins, since overexpression of β-arrestins has been shown to lead to internalization of receptors that previously were prevented from internalization due to resistance to phosphorylation or COOH-terminal truncation (32). One might speculate, assuming a similar mechanism for kinin receptor internalization, that overexpression of β-arrestin should also lead to internalization of WT B1 receptors.

Truncation of the cytoplasmic tail to Lys315 leads to a significant increase of the EC50 for total IP release determined after 30 min of stimulation. It is not yet clear whether this is because the cytoplasmic tail is directly involved in G-protein coupling or because this noninternalizing truncated receptor remains in the membrane in an active, but low affinity, state.

Desensitization of a receptor is generally demonstrated by a decrease in the amplitude of a response upon sequential stimulation with agonist. This approach is not meaningful for evaluating the B1KR, however, because, as we have previously reported, the B1KR agonist DAK has a very slow desensitization rate (7). Consequently, cells exposed to an initial stimulation with DAK that are then washed extensively and allowed to sit for an additional 10 min in buffer continue to exhibit a steady rise in IP upon a second incubation in buffer alone. This renders a repeat stimulation with DAK uninterpretable because of the limited number of unoccupied receptors available for binding. We chose, therefore, to compare the relative rates of accumulation of IP over time in the continued presence of agonist and were able by this method to demonstrate a clear difference in the rates of IP accumulation between B1KR and B2KR.

We expressed the data in these experiments, as in the EC50 calculations, as a percentage of “maximal” response, defined arbitrarily as the 30-min time point, rather than as Emax values. This was done, in part, because absolute increases in total IP varied significantly even within the same clones from experiment to experiment, presumably as a result of variability in cell densities, receptor numbers, and length of incubation with [3H]inositol. In addition, defining Emax (using a cumulative assay such as total IP) in a way that will allow meaningful comparison of a receptor that desensitizes and one that does not is difficult, if not impossible. Because the IP responses in B1KR and Lys315 do not plateau over the time points examined, defining maximal responses by an arbitrary time point is potentially misleading. Even in electing to use an arbitrary time (30 min) as “maximal” response, we are left with the additional problem of widely varying receptor densities among the clones examined. Although it has been reported that BK-stimulated IP production was not receptor number-dependent in cells possessing between 25,000 and 140,000 receptors/cell (34), we cannot confirm these data as yet for our own cell system.

The fact that both chimera, the two truncations of B2KR, and the point mutation C324A have dissociation constants at 0 °C that are similar to those of the wild type receptors indicates that the cytoplasmic tails play only a minor role in regulating ligand binding characteristics. As shown above, however, they play a significant role in signal termination. This is exemplified by the observation that truncation of the cytoplasmic tail of the B2KR to Lys315 results in a receptor that, like the B1KR, gets neither sequestered nor desensitized. It remains unclear, however, if this latter property of Lys315 and B1KR is due, in part, to the lack of a rapid, pronounced ligand-induced reduction in the number of available cell surface receptors or results from the absence of sequences that are important for desensitization by mechanisms other than removal of receptors from the cell surface. If such sequences do exist in the tail of the B2KR downstream of Cys324, their information apparently cannot be transferred, since the chimera B1CB2 does not show a desensitization pattern that resembles that of the B2KR (Fig. 5).

This study shows the importance of the COOH terminus as an independent domain for ligand-induced internalization of kinin receptors. The availability of chimeras of receptor subtypes that show completely different sequestration behavior but otherwise similar G-protein coupling and of an internalization-deficient mutant (Lys315) of B2KR should help us to gain greater understanding of the interaction of the different processes of receptor sequestration, signaling, and desensitization/
resensitization of kinin receptors in particular and, hopefully, of peptide receptors in general.

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REFERENCES

1. Regoli, D., and Barabe, L. (1988) Methods Enzymol. 163, 210–230
2. Prou, D., and Kaplan, A. (1988) Annu. Rev. Immunol. 6, 49–83
3. Bathon, J. M., and Prou, D. (1991) Annu. Rev. Pharmacol. Toxicol. 31, 129–162
4. Farmer, G. S., and Burch, R. M. (1992) Annu. Rev. Pharmacol. Toxicol. 32, 511–536
5. Figueroa, C. D., Worthy, K. (1992) Pharmacol Rev. 44, 1–80
6. Marceau, F. (1995) Immunopharmacology 30, 1–26
7. Austin, C. E., Faussner, A., Robinson, H. E., Sarvait, C., Kyle, D. J., Bathon, J. M., and Proud, D. (1997) J. Biol. Chem. 272, 11420–11425
8. Hess, F. J., Borkowski, J. A., Young, G. S., Strader, C. D., and Ransom, R. W. (1992) Biochem. Biophys. Res. Commun. 184, 260–268
9. Menke, J. G., Borkowski, J. A., Bierilo, K. K., McNeil, T., Derrick, A. W., Schneck, K. A., Ransom, R. W., Strader, C. D., Linemeyer, D. L., and Hess, J. F. (1994) J. Biol. Chem. 269, 21583–21586
10. Gudermann, T., Nuernberg, B., Schultz, G. J. (1995) J. Mol. Med. 73, 51–63
11. Trowbridge, I. S., Collawn, J. F., and Hopkins, C. R. (1993) Annu. Rev. Cell. Biol. 9, 129–161
12. Bohn, S. K., Khitin, L. M., Smeekens, S. P., Grady, E. F., Payan, D. G., and Bunnell, N. W. (1997) J. Biol. Chem. 272, 3236–32372
13. Ferguson, S. G., Downey, W. E., III, Colapietro, A.-M., Barak, L., Menard, L., Caron, M. G. (1996) Science 271, 363–366
14. Blaukat, A., Abd Alla, S., Lohse, M. J., and Muller-Esterl, W. (1996) J. Biol. Chem. 271, 32366–32374
15. Prado, G. N., Taylor, L., and Polgar, P. (1997) J. Biol. Chem. 272, 14638–14642
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