A Novel Mitogenic Signaling Pathway of Bradykinin in the Human Colon Carcinoma Cell Line SW-480 Involves Sequential Activation of a Gq/11 Protein, Phosphatidylinositol 3-Kinase β, and Protein Kinase Cε*

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The signaling routes connecting G protein-coupled receptors to the mitogen-activated protein kinase (MAPK) pathway reveal a high degree of complexity and cell specificity. In the human colon carcinoma cell line SW-480, we detected a mitogenic effect of bradykinin (BK) that is mediated via a pertussis toxin-insensitive G protein of the Gq/11 family and that involves activation of MAPK. Both BK-induced stimulation of DNA synthesis and activation of MAPK in response to BK were abolished by two different inhibitors of phosphatidylinositol 3-kinase (PI3K), wortmannin and LY 294002, as well as by two different inhibitors of protein kinase C (PKC), bisindolylmaleimide and Ro 31-8220. Stimulation of SW-480 cells by BK led to increased formation of PI3K lipid products (phosphatidylinositol 3,4,5-trisphosphate and phosphatidylinositol 3,4-bisphosphate) and to enhanced translocation of the PKCε isoform from the cytosol to the membrane. Both effects of BK were inhibited by wortmannin, too. Using subtype-specific antibodies, only the PI3K subunits p110β and p85, but not p110α and p110γ, were detected in SW-480 cells. Finally, p110β was found to be co-immunoprecipitated with PKCε. Our data suggest that in SW-480 cells, (i) dimeric PI3Kβε is activated via a Gq/11 protein; (ii) PKCε is a downstream target of PI3Kβε mediating the mitogenic signal to the MAPK pathway; and (iii) PKCε associates with the p110 subunit of PI3Kβε. Thus, these results add a novel possibility to the emerging picture of multiple pathways linking G protein-coupled receptors to MAPK.

MAPK subfamily, which includes the extracellular signal-regulated kinases Erk1 and Erk2, is stimulated via a consecutive activation of the protein kinases Raf and MEK. The MAPK cascade is initially switched on via activation of the low molecular mass GTP-binding protein Ras. GTP-bound Ras associates with the proximal kinase Raf to the plasma membrane, resulting in its activation.

Several signal transduction pathways from G protein-coupled receptors to MAPK have been proposed that may be classified according to the type of G protein involved (for review, see Refs. 1 and 2). Thus, MAPK activation via pertussis toxin (PTX)-sensitive G protein-coupled receptor, such as the m2 muscarinic receptor, is thought to be mediated by Gαi, subunits, phosphatidylinositol 3-kinase γ (PI3Kγ), and Ras (3). In contrast, receptors coupled to G proteins of the PTX-insensitive Gq/11 family, such as the m2 muscarinic receptor, mediate MAPK activation via a Gαi subunit that is Ras-independent and may involve PKC (4). Once activated, the different PKC isoforms, with the exception of PKCζ, activate the MAPK cascade at the level of Raf (5), but may also involve tyrosine kinases of the Src family (6, 7). MAPK activation by PTX-sensitive Gαi proteins appears to be independent of Gαi, and Ras, but requires PKC (8). Gαi-coupled receptors such as the β-adrenergic receptor were found to exert a dual effect on MAPK involving Gαi-mediated activation and cAMP-mediated inhibition (9). Alternatively, Ullrich and co-workers (10–12) have suggested an epidermal growth factor receptor transactivation by both Gαi- and Gq/11-coupled receptors as an essential prerequisite for MAPK activation. They propose an epidermal growth factor receptor tyrosine phosphorylation by G protein-coupled receptors as the key event, which might be mediated by cytosolic tyrosine kinases such as Src and PYK2.

In addition to receptor tyrosine kinases and PKC, PI3Ks appear to be key signaling enzymes implicated in the regulation of receptor-stimulated mitogen. After activation, they preferentially utilize phosphatidylinositol 4,5-bisphosphate as substrate, which is phosphorylated to phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P3), followed by rapid degradation to PtdIns(3,4)P2. Both molecules have been proposed to act as second messengers. Recent studies indicate that both PtdIns(3,4)P2 and PtdIns(3,4,5)P3 can directly activate certain PKC isoforms and the serine/threonine-protein kinase Akt/PKB (for review, see Refs. 13 and 14). In terms of mode of regulation, class I members are subdivided into receptor tyrosine kinase-associated (class Iα) or G protein-coupled receptor-activated (class Iβ) PI3Ks (for review, see Ref. 15). The class Iα types have been structurally characterized as a heterodimer consisting of a 110-kDa catalytic subunit (p110) and an 85-kDa

G protein-coupled receptors mediate effects of peptide hormones and neurotransmitters on intermediary metabolism as well as play an important role in the regulation of cell growth and differentiation. Similar to receptor tyrosine kinases, they initiate signaling pathways that finally activate members of the mitogen-activated protein kinase (MAPK) family. One

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1 The abbreviations used are: MAPK, mitogen-activated protein kinase; PTX, pertussis toxin; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PtdIns(3,4)P2, phosphatidylinositol 3,4-bisphosphate; PtdIns(3,4,5)P3, phosphatidylinositol 3,4,5-trisphosphate; PKCε, bradykinin; PVDF, polyvinylidene difluoride; CTX, cholera toxin; BSA, bovine serum albumin; TES, 2-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl] amino/ethanesulfonic acid; MOPS, 4-morpholinepropanesulfonic acid.
regulatory subunit (p85). They are stimulated through receptors with intrinsic or associated tyrosine kinase activity that bind to the p85 subunit, thereby inducing PI3K activity. The only known class I IA and IB members was questioned very recently 

![p85/p110](image)

in vitro, PI3K has been shown to respond synergistically to both G-protein, and a synthetic phosphotyrosyl peptide that binds to the SH2 domain of p85 (19). These and other studies (20, 21) suggest that also a p85/p110 PI3K may be regulated in the downstream region of pertussis toxin-sensitive G proteins.

In this report, we present evidence for the activation of p85/p110β by the G protein-coupled bradykinin receptor in intact human colon carcinoma SW-480 cells. In addition, we obtained results showing that protein kinase Ce is a mediator connecting PI3K with the MAPK signaling cascade in this endothelial cell line.

**EXPERIMENTAL PROCEDURES**

**Materials**—[3,4-3H2]Pro3,4[Bradykinin (102 Ci/mmol), myo-[2-3H]inositol (205 Ci/mmol), γ-[32P]ATP (3000 Ci/mmol), and 32P-labeled myelin basic protein were purchased from AMERISHAM Pharmacia Bio-RAD. Radioactivity of the inositol phosphate-containing fractions was determined by liquid scintillation counting.

**Measurement of p44 MAPK (Erk1) Activity**—SW-480 cells were preincubated in serum-free RPMI 1640 medium for 2 h and then treated with the different inhibitors and/or BK as indicated in the figure legends. After stimulation, cells were scraped off and centrifuged for 1 min at 5000 × g. The medium was removed, and the pellets were lysed in 1 ml of lysis buffer (20 mM HEPES, pH 7.5, 10 mM EDTA, 40 mM β-glycerophosphate, 1% Triton X-100, 2.5 mM MgCl2, 2 mM orthovanadate, 1 mM diithioctreitol, 1 mM phenylmethylsulfonyl fluoride, 20 µg/ml aprotinin, and 20 µg/ml leupeptin). After a 30 min incubation on ice, the lysates were centrifuged (15,000 × g, 4 °C) to pellet insoluble material. The supernatants were transferred into new tubes, and Erk1 was immunoprecipitated using a rabbit polyclonal antibody (1 µg/ml of lymphocyte from Santa Cruz Biotechnology). The immunoprecipitates were subsequently washed with phosphate-buffered saline containing 1% Triton X-100 and 2 mM orthovanadate; Tris-HCl, pH 7.5, containing 0.5 mM phenylmethylsulfonyl fluoride, 1 mM β-glycerophosphate, 7.5 mM MgCl2, 0.5 mM EDTA, 0.5 mM sodium fluoride, and 0.5 mM orthovanadate. Phosphorylation of immunoprecipitates was performed in 30 µl of kinase buffer supplemented with 1 µCi of [γ-32P]ATP, 20 µM ATP, 1.5 mg/ml myelin basic protein, and 3.3 µM diithioctreitol. After 20 min at 30 °C, the reaction was terminated by the addition of 10 µl of SDS-polyacrylamide gel electrophoresis buffer. The samples were analyzed by SDS-polyacrylamide gel electrophoresis on 12% (w/v) gels. The dried gels were autoradiographed, and the radioactivity incorporated into myelin basic protein was quantified using a PhosphorImager (NIH Image Version 1.57).

**PKC Translocation**—For the measurement of PKC translocation, SW-480 cells were subjected to serum-free RPMI 1640 medium for 2 h before stimulation. The cells were then exposed to BK (100 nM) for 5 min at 37 °C. For several experiments, cells were pretreated with the PI3K inhibitor wortmannin for 30 min. The incubation was terminated by removing the cells and centrifuging at 20,000 × g for 1 min at 4 °C. The pellets were resuspended in 50 mM HEPES, pH 7.4, containing bacitracin (100 µg/ml), phenylmethylsulfonyl fluoride (0.1 mM), pepstatin A (1 µg/ml), and leupeptin (2 µg/ml) and were stored at −80 °C. Protein concentration was determined according to Bradford (22).
tibody (1 µg/ml) at 4 °C for 2 h on a rotating drive. Antigen-antibody complexes were recovered using protein A-Sepharose. The immunoprecipitates were washed three times with phosphate-buffered saline, pH 7.4, containing 1% Triton X-100 and 2 mM vanadate; resuspended in 50 µl of electrophoresis sample buffer, boiled for 5 min; and subjected to SDS-polyacrylamide gel electrophoresis using a 7.5% gel, followed by transfer to PVDF membranes. After blocking overnight with 3% nonfat milk in Tris-buffered saline and 0.5 M NaCl, PVDF blots were incubated with the appropriate primary antibodies (Santa Cruz Biotechnology), and horseradish peroxidase-conjugated anti-rabbit IgG was used for detection with the ECL system.

32P labeling of SW-480 cells and analysis of phosphatidylinositol phosphates—PI3K lipid kinase activity was determined using the method of Stephens et al. (20) with minor modifications. Briefly, SW-480 cells were freshly isolated; washed two times with phosphate-free RPMI 1640 medium; and incubated for 1 h in phosphate-free RPMI 1640 medium containing 25 mM HEPES, pH 7.5, 1 mg/ml fatty acid-free BSA, and 10% fetal calf serum. The SW-480 cells were then labeled overnight with 100 µCi of [32P]inositol (6 × 106 cells/2 ml). After labeling, cells were washed two times with 140 mM NaCl, 5 mM KCl, 2.8 mM CaCl2, 1.5 mM MgCl2, 0.066 mM MgSO4, 15 mM HEPES, 5.6 mM glucose, and 0.1% BSA, pH 7.2, at 37 °C; centrifuged at 1200 rpm for 5 min; resuspended in 0.5 ml of the above buffer; and treated with PKC inhibitor as indicated. Reactions were terminated by the addition of 1 ml of ice-cold 2.4 M HCl. Then, 1 ml of chloroform/methanol/HCl (1:2:1), 0.75 ml of chloroform/phosphoinositol mixture (with 10 µg of phosphoinositol mixture/point; Sigma), and 1 ml of chloroform were added subsequently. The mixture was thoroughly vortexed, and phase separation was performed by a short centrifugation (2500 rpm, 4 min). The lower chloroform phase was transferred to a new vial, and the upper phase was re-extracted twice with 1.5 ml of chloroform. Pooled chloroform phases were dried, and the lipids were decacylated by incubation for 1 h in methanolamine (33% v/v) in ethanol; Fluuka) at 50 °C. After removal of the methanolamine, the samples were resuspended in 1 ml of water and extracted twice with 1 ml of 1-butanol. The aqueous phase containing the labeled lipid head group was analyzed by high pressure liquid chromatography as described (24).

RESULTS

Bradykinin B2 Receptor-mediated Mitogenic Effects in SW-480 Cells—In the human colon carcinoma cell line SW-480, we detected an endogenously expressed bradykinin receptor. Binding studies with [3H]BK (displacement experiments) revealed an IC50 value of ~1 nM (Fig. 1A). After prelabeling of SW-480 cells with myo-[3H]inositol, BK induced a concentration-dependent increase in inositol phosphate formation with an EC50 value of ~3 nM (Fig. 1B). The phosphatidylinositol system represents the main signaling pathway of bradykinin B2 receptors in most tissues or cells (25). Both the binding parameter and the dose-response curve are in good agreement with those for other B2 receptors (25). As in small cell lung cancer cells (26), BK exerted a mitogenic effect in SW-480 cells as measured with the thymidine incorporation assay. This effect of BK was completely blocked in the presence of the non-peptidic bradykinin B2 receptor antagonist FR 173657 (27), suggesting the involvement of the B2 receptor subtype in the mitogenic action of BK (Fig. 1C).

Bradykinin-induced Cell Proliferation Is Mediated via the Extracellular Signal-regulated Protein Kinase/MAPK Pathway—Treatment of SW-480 cells with BK led to the immediate activation of p44 MAPK as determined using the myelin basic protein assay (Fig. 2). To investigate whether activation of the MAPK pathway is required for the induction of cell division by BK, we investigated the effect of BK on thymidine incorporation in the presence of PD 098059, which inhibits the activation of MAPK by blocking the activity of MAPK kinase (MEK) (28). Under the conditions used, both the BK-induced cell proliferation and the MAPK activation by BK were completely abolished in the presence of PD 098059 (Fig. 2). It may be concluded that the proliferation of SW-480 cells in response to BK is dependent on the activation of the MAPK pathway.

Effects of CTX or PTX on MAPK Activation in Response to Bradykinin—In SW-480 cells, the BK-induced MAPK activation was insensitive to treatment with PTX (200 ng/ml) (Fig. 3A). The same PTX concentration was shown to effectively inhibit MAPK activation by lysophosphatidic acid in PC-12 cells (29).

Besides Gs and Gq/11 proteins, immunoblotting experiments with specific antibodies (Santa Cruz Biotechnology) revealed the presence of Gαs, Gα12, and Gα13 proteins, whereas Gα1 and Gαz were not detected in SW-480 cells (data not shown). To investigate whether the PTX-insensitive G protein might play a role in the mitogenic signaling pathway of BK, SW-480 cells were treated with CTX. The effect of BK on MAPK activity was clearly abolished by CTX (Fig. 3B). In addition, treatment of SW-480 cells with forskolin also prevented the activation of MAPK by BK (Fig. 3C). Since cAMP has been reported to inhibit MAPK in smooth muscle cells and some fibroblast cell lines (30, 31), we conclude that the permanent activated adenylate cyclase in the presence of CTX counteracts the stimulation of MAPK activity in response to BK.

Effects of BK on DNA Synthesis and MAPK Are Blocked by Both Inhibitors of PI3K and PKC—Next we tested two different inhibitors of PI3K, wortmannin and LY 294002, for their ability to affect the mitogenic action of BK in SW-480 cells. When PI3K was blocked, neither DNA synthesis (Fig. 4) nor MAPK activity (Fig. 5) was stimulated by BK, suggesting an involvement of a PI3K in the BK signaling pathway in SW-480 cells. Furthermore, two different inhibitors of PKC, bisindolylmale-
imide and Ro 31-8220, were used to study the involvement of PKC in the mitogenic action of BK in SW-480 cells. As shown in Figs. 4 and 6, also in the presence of PKC inhibitors, BK failed to induce both stimulation of DNA synthesis and activation of MAPK, suggesting an involvement of protein kinase C in the mitogenic signaling pathway of BK in SW-480 cells as well. Taken together, these results obtained with different inhibitors and different experimental approaches indicate that a PI3K as well as a PKC are downstream mediators of the Gq protein-coupled bradykinin receptor in SW-480 cells.

Fig. 3. Effects of PTX, CTX, and forskolin on BK-induced activation of MAPK. A, SW-480 cells were preincubated with PTX (200 ng/ml) for 24 h. The effects of PTX treatment on basal and BK-stimulated MAPK activities was determined after a 5-min exposure to BK (100 nM) or LY 294002 (100 nM) and with the PKC inhibitors bisindolylmaleimide (BFS; 5 μM) and Ro 31-8220 (Ro; 5 μM). Thereafter, BK (10 nM) was added for 36 h and [3H]thymidine for 12 h of incubation. The rate of DNA synthesis was determined as described under “Experimental Procedures.” The results represent the means ± S.E. of 12 wells representative of two independent experiments. *, significantly different from the control; **, significantly different compared with the BK effect in the absence of the inhibitor (p < 0.05; Student’s test).

Intact SW-480 Cells—In SW-480 cells prelabeled with [32P]Pi, BK rapidly stimulated the accumulation of PtdIns(3,4,5)P3 and PtdIns(3,4)P2 (Fig. 7). The levels of [32P]PtdIns(3,4,5)P3 and of its metabolite, [32P]PtdIns(3,4)P2, reached a maximum after 8–15 s and decreased after 1 min (data not shown). The quantity of accumulated [32P]PtdIns(3,4,5)P3 and of its metabolite, [32P]PtdIns(3,4)P2, is comparable to their pattern of accumulation in human neutrophils after stimulation with fMet-Leu-Phe (20). In SW-480 cells pretreated with wortmannin, BK failed to stimulate lipid kinase activity.

SW-480 Cells Contain p58/p110 PI3Kb—To investigate which subtype of class I PI3Ks may be activated by BK we analyzed SW-480 cell lysates by Western blotting using specific antibodies against the catalytic subunits p110α, p110β, and p110γ and against the regulatory subunits p85α and p85β. Fig. 8 shows that in SW-480 cells, only p110β and the p85α and p85β subunits exhibited significant expression, whereas p110α and p110γ were not detectable by immunoblotting. Thus, heterodimeric PI3Kb, but not monomeric PI3Kγ, appears to be the target of the bradykinin receptor-stimulated Gq protein.
**PKCe May Be a Mediator Connecting PI3Kβ with the MAPK Cascade**—Among the different PKC isoforms, the novel PKCe, PKCd, and PKCγ as well as the atypical PKCe and PKCβII have been demonstrated to be activated by PtdIns(3,4,5)P3 and/or PtdIns(3,4)P2 in vitro (32, 33). Western blotting of whole cell extracts established that SW-480 cells express the PKC isoforms ε, δ, and γ. For activation studies, we measured the stimulus-induced translocation of PKCe from the cytosol to the plasma membrane. Following the kinetics of BK-induced translocation of PKCe isoforms in other cells (29, 33) SW-480 cells were stimulated with 100 nM BK for 5 min. Throughout the repeated experiments, only PKCe showed an increased membrane association when cells were triggered with BK. The BK-induced translocation of PKCe was completely abolished in the presence of wortmannin (Fig. 9), suggesting that activation of PKCe is a downstream event of the BK-induced activation of PI3Kβ. The mechanism whereby PKCe isoforms may be activated by PI3K in vivo is not yet clear. Recently, a specific association (co-immunoprecipitation) of PKCe with PKCd after cytokine stimulation in human erythroleukemia cells was reported (34). Therefore, we examined a possible association of PKCe with p110β. Cell lysates from SW-480 cells were immunoprecipitated with anti-PKCε antibodies and analyzed with antibodies to p110β. Indeed, PKCe and PKCd were found to co-immunoprecipitate in SW-480 cells in a specific manner as demonstrated by control experiments with non-immune serum (Fig. 10). There was no detectable increase in association of PKCe and PI3Kβ in BK-treated cells (data not shown).

**DISCUSSION**

In this study, we investigated the signaling pathway linking the endogenously expressed bradykinin receptor to MAPK in the human colon carcinoma cell line SW-480. We present evidence for the activation of p85/p110β PI3K downstream of the bradykinin B2 receptor, which couples to a PTX-insensitive G protein. To our knowledge, this is the first demonstration that (i) a tyrosine kinase-associated PI3K is activated by a G protein-coupled receptor solely in an intact cell system and that (ii) the activation of PI3K is a downstream event of the BK-induced activation of G protein. The mechanism whereby PKCe isoforms may be activated by PI3K in vivo is not yet clear. Recently, a specific association (co-immunoprecipitation) of PKCe with PKCd after cytokine stimulation in human erythroleukemia cells was reported (34). Therefore, we examined a possible association of PKCe with p110β. Cell lysates from SW-480 cells were immunoprecipitated with anti-PKCε antibodies and analyzed with antibodies to p110β. Indeed, PKCe and PKCd were found to co-immunoprecipitate in SW-480 cells in a specific manner as demonstrated by control experiments with non-immune serum (Fig. 10). There was no detectable increase in association of PKCe and PI3Kβ in BK-treated cells (data not shown).

**FIG. 6.** Activation of MAPK in SW-480 cells is inhibited by bisindolylmaleimide and Ro 31-8220. SW-480 cells were pretreated with either 30 μM Ro 31-8220 (A) or 5 μM bisindolylmaleimide (B) for 30 min, followed by the addition of BK (100 nM) for 5 min. The cells were then lysed, and MAPK activity was determined as described under “Experimental Procedures.” Shown are autoradiograms that are representative of the results obtained in three to four separate experiments. **MBP**, myelin basic protein.

In contrast to the hitherto existing idea that PI3K exclusively mediates the effect of βγ-complexes released from G1 proteins, the G protein involved in SW-480 cells is PTX-insensitive. Among the PTX-insensitive G proteins expressed in SW-480 cells, G12/13 do not stimulate phosphatidylinositol hydrolysis (36) and may be excluded from linking the bradykinin receptor to phospholipase Cβ. The bradykinin receptor appears to be capable of interacting with multiple G proteins, including also Gs (23, 37). If the effect of bradykinin on MAPK is triggered by βγ-complexes released from a G protein as demonstrated for the β-adrenergic receptor (9), it might be expected that permanent activation of Gs in the presence of CTX stabilizes or potentiates the BK action on MAPK. Surprisingly, treatment of SW-480 cells with CTX completely prevented the activation of MAPK induced by BK. Furthermore, the BK-induced activation of MAPK was abolished in the presence of forskolin, which activates adenylate cyclase independently of the Gs protein. It may therefore be assumed that the inhibitory effect of CTX on the BK-induced stimulation of MAPK activity is due to cAMP triggered by CTX. We conclude that the G protein involved in both stimulation of phospholipase Cβ by BK and stimulation of MAPK in response to BK belongs to the G11 family.

**Our results suggest the involvement of a PKCe upstream or downstream of PI3Kβ. One plausible candidate to play a role as a downstream effector of PI3K is PKCe since PKCe is activated by both lipid-derived second messengers of PI3K, PtdIns(3,4,5)P3 and PtdIns(3,4)P2 (33, 38). Overexpression of PKCe, but not that of PKCd, another target of PI3K, has been shown to induce cell transformation (39) as well as activation of Raf-1 kinase (40) and MAPK (5). Both PKCd and PKCe were found to associate with PI3K in TF-1 cells, a human erythroleukemia cell line (41). In addition, PKCe was suggested to be a mediator connecting PI3K with the MAPK pathway in erythroid progenitor cells (42).

We obtained two lines of evidence indicating a link between PKCe and PI3K in SW-480 cells. First, BK-induced translocation of PKCe is sensitive to wortmannin, and second, PKCe associates with p110β as demonstrated by co-immunoprecipitation. This association was not enhanced after stimulation of SW-480 cells with bradykinin. Similarly, in TF-1 cells, only the association of PI3K with PKCd, but not that with PKCe, was
found to be increased after cytokine stimulation (41). There are also contradictory results whether or not PI3K lipid products may be a prerequisite for the PI3K/PKC association. In TF-1 cells, wortmannin inhibited this association, whereas LY 294002 did not (41). In our case, the inhibitory effect of wortmannin on the BK-induced translocation of PKCε probably mediates activation of PI3Kβ (19). By an unknown mechanism, PI10β recruits and activates PKCε, which presumably precedes activation of Raf kinase (5) and, subsequently, MAPK (Fig. 11).

Fig. 7. Effect of bradykinin on accumulation of PtdIns(3,4,5)P3 and PtdIns(3,4)P2 in intact SW-480 cells. 32P-labeled human colon carcinoma SW-480 cells were challenged with 100 nM BK, and the levels of [32P]PtdIns(3,4,5)P3 (A) and [32P]PtdIns(3,4)P2 (B) were determined after 15 s as described under “Experimental Procedures.” Data shown are mean cpm obtained from two or three independent experiments. *, significantly different from the control (p < 0.05). For some experiments, SW-480 cells were pretreated for 30 min with 100 nM wortmannin (Wo) before bradykinin was added.

Fig. 8. Endogenous expression of PI3K subunits in SW-480 cells. Lysates from SW-480 cells were subjected to Western blot analysis using specific antibodies to p110β, p110γ, p85α, and p85β. No significant immunoreactivity was detected with antibodies to p110α and p110γ.

Fig. 9. Effect of wortmannin on bradykinin-stimulated translocation of PKCε. SW-480 cells were exposed to 100 nM BK for 10 min in the absence or presence of wortmannin (100 nM, 30-min preincubation). Membranes were prepared and analyzed by immunoblotting with antisera to the different PKC isofoms indicated (1 μg/ml) as described under “Experimental Procedures.” Representative immunoblots are shown after background smoothing and quantification with the program NIH Image Version 1.57 of experiments repeated three times with similar results.

Fig. 10. Anti-PKCε immunoprecipitates from SW-480 cells co-immunoprecipitated p110β PI3K. Cell lysates from SW-480 cells were immunoprecipitated with anti-PKCε antibody and analyzed by immunoblotting with antibody to PKCε (lane A) or anti-p110β antibody (lane C). Lane B shows an immunoprecipitate with non-immune serum Western blotting with anti-p110β antibody. Lane D shows a control blot with p110β-glutathione S-transferase fusion protein (135 kDa), re-probed with anti-p110β antibody.

Fig. 11. Model of bradykinin receptor-mediated PI3Kβ- and PKCε-dependent MAPK activation in SW-480 cells. Activation of a Gq11 protein in response to BK leads to release of Gβγ complexes, which probably mediate activation of PI3Kβ (19). By an unknown mechanism, p110β recruits and activates PKCε, which presumably precedes activation of Raf kinase (5) and, subsequently, MAPK. BKR, BK receptor.

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