Mammalian bombesin receptors are coupled to multiple signal transduction pathways in pancreatic acini

HIROKAZU NISHINO, YASUHIRO TSUNODA, AND CHUNG OWYANG
Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan 48109

Nishino, Hirokazu, Yasuhiro Tsunoda, and Chung Owyang. Mammalian bombesin receptors are coupled to multiple signal transduction pathways in pancreatic acini. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G525–G534, 1998.—We investigated the structural requirements for bombesin (BB)-like peptides to stimulate amylase secretion in rat pancreatic acini and examined the responsible intracellular signal transduction pathways. The tetradecapeptide BB-(1—14) was a full agonist, whereas the heptapeptide BB-(8—14) did not evoke amylase secretion. The mammalian BB analog neurenomed C decapptide [NMC-(5—14)] was as potent as BB-(1—14) in stimulating amylase secretion, suggesting that Gly5—Asn6—His7 (or Gln7) of the COOH-terminal decapptide are essential amino acids for full biological activity. BB and NMC equipotently stimulated d-myoinositol 1,4,5-trisphosphate production, which was inhibited by the phospholipase C (PLC) inhibitor U-73122. BB and NMC also stimulated protein tyrosine kinase (PTK) activities. The half-maximal effective concentration (EC50) for NMC-activated PTK was 2 log units less than the EC50 for BB-activated PTK. NMC was 10–34 times more potent than BB in increasing leukotriene C4 (an index of arachidonic acid production). The production of leukotriene C4 was inhibited by the phospholipase C inhibitor ONO-RS-082. BB and NMC equipotently stimulated 1,4,5-trisphosphate production, which was inhibited by the PLC inhibitor U-73122. BB and NMC also stimulated protein tyrosine kinase (PTK) activities. The half-maximal effective concentration (EC50) for NMC-activated PTK was 2 log units less than the EC50 for BB-activated PTK. NMC was 10–34 times more potent than BB in increasing leukotriene C4 (an index of arachidonic acid production). The production of leukotriene C4 was inhibited by the phospholipase C inhibitor ONO-RS-082. BB and NMC equipotently stimulated d-myoinositol 1,4,5-trisphosphate production, which was inhibited by the PLC inhibitor U-73122.

METHODS

Materials. Human GRP, porcine NMC, porcine NMB, and neurenomed K (NMK) were obtained from Sigma Chemical (St. Louis, MO). BB-(1—14), BB-(8—14), BB-(9—14), and BB-(11—14) were from Research Plus (Bayonne,
benzoic acid) were purchased from Biomol (Plymouth Meeting, PA). Genistein (4',5',7-trihydroxyisoflavone) was obtained from Gibco-BRL (Grand Island, NY), and Fura 2-AM was from Molecular Probes (Eugene, OR).

Isolation of pancreatic acinar cells and measurements of intracellular Ca2+ concentration and amylose secretion. Isolated rat pancreatic acini were prepared by collagenase digestion with pancreata obtained from male Sprague-Dawley rats (40). Acini were suspended in a physiological salt solution (PSS). The PSS contained 0.1% bovine serum albumin, 0.1 mg soybean trypsin inhibitor, and in (mM) 137 NaCl, 4.7 KCl, 0.56 MgCl2, 1.28 CaCl2, 1 Na2HPO4, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), Eagle's minimal essential amino acid neutralized (HEPES), and 5.5 D-glucose and was adjusted to pH 7.35 and equilibrated with 100% O2.

The intracellular Ca2+ concentration ([Ca2+]i) measurements in individual pancreatic acini were performed as described previously (40). In brief, isolated acini were incubated with 2 µM fura 2-AM at 37°C in 10 ml PSS for 30 min. All experiments were done using a dual excitation wavelength (340/380 nm emitted at 505 nm) in a modular fluorometer system (SPEX) coupled to a Nikon Diaphot inverted microscope (×40). Isolated acini were placed on a cover glass and mounted on a closed chamber were superfused from a reservoir (1 ml/min). A fluorescence ratio was converted to [Ca2+]i according to in vitro calibration with an external standard and 25 µM fura 2 potassium salt.

Amylose secretion studies were performed using acini that were preincubated for 30 min in 40 ml PSS, washed twice by centrifugation (5000 rpm) for 3 min, and resuspended in 40 ml fresh PSS. Aliquots were distributed into flasks and incubated with reagents for 60 min at 37°C. The incubation was terminated by centrifugation (10,000 revolutions/min (rpm)) for 30 s at 4°C in a Microfuge (1 ml × 2 in 20 groups). The amylase released into the supernatant and remaining in the pellet was assayed, using Procion yellow starch as a substrate. Amylase secretion was expressed as the percentage of the total content in each sample.

Measurement of IP3. The radioimmunoassay (RIA) of IP3 was performed as previously described (IP3 assay system; Amersham, Arlington Heights, IL) (20). In brief, 0.5-ml aliquots of the acinar suspension (2 × 106 cells/PSS) were incubated with reagents at 37°C for various time intervals. Incubation was stopped by adding chilled trichloroacetic acid (TCA) (0.125 ml) to obtain a final TCA concentration of 10%. After sonication for 30 s and allowing the cell suspension to settle for 30 min at 4°C, whole fractions were centrifuged (10,000 rpm) for 10 min at 4°C. The resultant supernatant (100–150 µl) was extracted with 1 ml chloroform, dried under a nitrogen stream, and stored at −20°C. Each suspension was thawed and sonicated for 30 s at 4°C. The sonicates were vortexed and allowed to settle for 10 min at 4°C and centrifuged (10,000 rpm) for 15 min at 4°C. Supernatants (10-µl samples, 50 µg protein) were incubated in 12.5 µl of substrate solution containing 1 mM RR-SRC peptide substrate (29). The substrate solution also contained 60 mM HEPES, 20 mM MgCl2, 40 µM EDTA, 0.2 mM DTT, 50 µg/ml bovine serum albumin, 0.3% (vol/vol) Nonidet P-40, 140 µM sodium orthovanadate, 120 µM ATP, and 1 µCi of [γ-32P]ATP. For the control reactions, supernatants (10-µl samples of cell extracts) were incubated with 12.5 µl substrate solution without RR-SRC peptide. After incubation for 30 min at 37°C, the reaction was stopped with 20 µl of ice-cold 10% TCA. All samples were placed on ice for 10 min and then centrifuged (10,000 rpm) for 10 min at 4°C. Supernatant (21.25 µl from each tube) was removed and spotted onto separate phosphocellulose disks. Each disk was placed in a scintillation vial containing 10 µl of 5% acetic acid. Subsequent to mixing at room temperature for 10 min, the washing reagent was decanted and each paper was mixed with 10 ml of 1% acetic acid for 10 min followed by 10 ml distilled water for 10 min at room temperature after decanting 1% acetic acid. After the distilled water was decanted, 10 ml of scintillant (Cytoscin) were added to each vial and the radioactivity remaining on each spotted paper was counted in a liquid scintillation counter. Nonspecific binding of [32P]ATP to the binding paper without the substrate was subtracted from each control sample. PTK activities were expressed as picomoles per minute per milligram protein of the cell extract.

Measurements of leukotriene C4. Leukotriene C4 (LTC4) was measured by RIA using the LTC4-specific 3H assay system (Amersham). Aliquots (0.5 ml) of the acinar cell suspension (2 × 106 cells/PSS) were incubated with reagents at 37°C for various time intervals. At each time interval, the incubation was stopped with 1 ml chilled PSS and suspensions were immediately centrifuged (10,000 rpm) for 40 s at 4°C in a Microfuge. The supernatant was removed, and the resultant pellet was resuspended in 50 µl chilled 50 mM HEPES buffer (pH 7.4) containing (in mM) 50 β-glycerocephosphate, 25 NaF, 150 NaCl, 20 ethylene glycol bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), 15 MgCl2, 1 dithiothreitol (DTT), 1% Triton X-100, 25 µg/ml leupeptin, and 25 µg/ml aprotinin. The suspension was immediately frozen in liquid nitrogen and stored at −70°C overnight. Each suspension was thawed and sonicated for 30 s at 4°C. The sonicates were vortexed and allowed to settle for 10 min at 4°C and then centrifuged (10,000 rpm) for 15 min at 4°C. Supernatants (10-µl samples, 50 µg protein) were incubated in 12.5 µl substrate solution without RR-SRC peptide. After incubation for 30 min at 37°C, the reaction was stopped with 20 µl of ice-cold 10% TCA. All samples were placed on ice for 10 min and then centrifuged (10,000 rpm) for 10 min at 4°C. Supernatant (21.25 µl from each tube) was removed and spotted onto separate phosphocellulose disks. Each disk was placed in a scintillation vial containing 10 µl of 5% acetic acid. Subsequent to mixing at room temperature for 10 min, the washing reagent was decanted and each paper was mixed with 10 ml of 1% acetic acid for 10 min followed by 10 ml distilled water for 10 min at room temperature after decanting 1% acetic acid. After the distilled water was decanted, 10 ml of scintillant (Cytoscin) were added to each vial and the radioactivity remaining on each spotted paper was counted in a liquid scintillation counter. Nonspecific binding of [32P]ATP to the binding paper without the substrate was subtracted from each control sample. PTK activities were expressed as picomoles per minute per milligram protein of the cell extract.
Centrifugation (10,000 rpm) for 3 min at 4°C with 0.5 ml dextran-coated charcoal in phosphate-buffered saline. The supernatant was solubilized in 10 ml scintillant (Cytoscint), and the radioactivity was counted in a liquid scintillation counter. LTC₄ levels were expressed as picograms per milligram protein of the cell extract. LTC₄ has little cross-reactivity with other arachidonic acid (AA) metabolites: 5% with LTD₄, 0.5% with leukotriene E₄, and <0.001% with other metabolites.

**RESULTS**

Evidence that Gly⁵-Asn⁶-His⁷ (or Gln⁷) of BB-like peptides are key amino acids for full biological activity. The structure of the BB analogs used in this study is shown in Fig. 1. The basal amylase secretion was 9.2 ± 0.3% of total/60 min (n = 31) in dispersed rat pancreatic acini. As shown in Fig. 2, frog BB, porcine NMC, human GRP, and porcine NMB each caused a dose-dependent and monophasic amylase secretion with peak increases of 3.2- to 3.6-fold over basal at the supramaximal dose (100 nM). The half-maximal effective concentrations (EC₅₀) for BB, NMC, GRP, and NMB were 0.2, 0.1, 0.2, and 3 nM, respectively. The tetradecapeptide BB-(1—14) was a full agonist, whereas the heptapeptide BB-(8—14), which has the same COOH-terminal seven amino acids as BB, GRP, and NMC, did not evoke amylase secretion at any dose (1 pM—100 nM). This indicates that the COOH-terminal heptapeptide is not sufficient for biological activity. The shorter BB peptides [e.g., BB-(9—14) and BB-(11—14)] did not increase amylase secretion over basal. Because the BB receptor has sequence similarity with the tachykinin receptor (19, 34), effects of NMK (neurokinin B), which is an agonist of tachykinin receptors, were examined. NMK did not evoke amylase secretion, suggesting the absence of tachykinin receptors in pancreatic acini. However, the decapeptide NMC-(5—14) was as potent as BB-(1—14) in stimulating amylase secretion, suggesting that Gly⁵-Asp⁶-His⁷ (or Gln⁷) of the BB-like peptides are critical amino acids for full biological activity.

BB and NMC increase IP₃ levels, PTK activities, and AA metabolite production. We further investigated signal transduction pathways used by the nonmammalian and mammalian BB analogs. The basal IP₃ was 1.50 ± 0.95 pmol/mg protein (n = 8). BB (10 nM, nonmammalian) and NMC (100 nM, mammalian) equipotently stimulated intracellular IP₃ production with a peak increase of 5.8 (n = 8) and 5.3-fold (n = 4) over basal, respectively, after 15 s of cell stimulation. These responses induced by BB and NMC (10–100 nM) were abolished by pretreatment of acini with the PLC inhibitor U-73122 (5 µM) [2.30 ± 1.29 pmol/ng protein for BB (n = 4) and 0.95 ± 0.48 pmol/ng protein for NMC (n = 4)].

BB and NMC dose dependently stimulated PTK activities with peak increases of 3.8-fold over basal after 3 min of cell stimulation. Basal PTK activity was 0.25 ± 0.06 pmol·min⁻¹·mg protein⁻¹ (n = 6) (Fig. 3A). The EC₅₀ for NMC-activated PTK was 2 log units less than the EC₅₀ for BB-activated PTK. Peak PTK levels for both BB (100 nM) and NMC (100 nM) were attained after 1 min and sustained for up to 10 min after cell stimulation (Fig. 3B).

LTC₄ and 15-hydroxyeicosatetraenoic acid (HETE), but not prostaglandins, are major metabolites of AA in pancreatic acini (38). They are produced by activation of the 15-lipoxygenase pathway (2). RIA provides a more quantitative measurement than direct release studies of AA. For these reasons, we measured intracellular LTC₄ levels ([LTC₄]i) as an index of AA production. Basal [LTC₄] was 23.23 ± 16.88 pg/mg protein (n = 8). As shown in Fig. 4A, BB (30 nM) and NMC (30 nM) stimulated [LTC₄] with increases of 5.0- and 38.1-fold over basal, respectively, after 3 min of cell stimulation. These increases in [LTC₄] induced by BB and NMC were significantly inhibited by pretreatment of acini with the PLA₂ inhibitor ONO-RS-082 (10 µM) for 10 min. Our time course studies showed that NMC (100 nM) was 10–34 times more potent than BB (100 nM) in
increasing \([\text{LTC}_4]\) at various time intervals (Fig. 4B), suggesting that NMC, the mammalian type of BB analog, preferentially activates the \(\text{PLA}_2\) pathway to produce AA and its metabolites.

Effects of inhibitors of PLC, PTK, and \(\text{PLA}_2\) on \(\text{Ca}^{2+}\) spiking induced by BB and NMC.

As described in the previous section, BB and NMC equipotently stimulated IP\(_3\) production in pancreatic acini. To further investigate the biological significance of the BB receptor-coupled PLC-IP\(_3\) pathway, we examined the effects of PLC inhibitor on \(\text{Ca}^{2+}\) spiking stimulated by BB and NMC in fura 2-loaded individual rat pancreatic acini. Reports have shown that in rabbit pancreatic acinar cells, the PLC inhibitor U-73122 (3–10 \(\mu\)M) alone caused an increase in \([\text{Ca}^{2+}]\) (46). However, in individual rat pancreatic acini, application of U-73122 (5 and 10 \(\mu\)M) for 5–10 min caused no change in basal \([\text{Ca}^{2+}]\) in six of six cells (Fig. 5A, inset). Similarly, application of the PTK inhibitor genistein (100 \(\mu\)M) and the \(\text{PLA}_2\) inhibitor ONO-RS-082 (10 \(\mu\)M) also did not change basal \([\text{Ca}^{2+}]\) in nine of nine cells. U-73122, genistein, and ONO-RS-082 were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 0.01–0.1%. At these concentrations, DMSO did not produce a change in \([\text{Ca}^{2+}]\). U-73122 (5 \(\mu\)M) transiently inhibited the sustained \([\text{Ca}^{2+}]\) increase (or \(\text{Ca}^{2+}\) oscillations), but this was not inhibited by U-73122 in four of five cells. The sustained \([\text{Ca}^{2+}]\) plateau induced by higher concentrations of BB...
eliminated the sustained plateau and Ca\(^{2+}\) oscillations induced by low doses of BB (0.1–10 nM) in 75% of cells examined (6 of 8 cells) (Fig. 6A). Genistein (100 µM) eliminated the sustained \([Ca^{2+}]_i\) plateau evoked by high doses of BB (100 nM) in three of six cells (Fig. 6B). In separate studies, we showed that genistein inhibited NMC (0.3–1 nM)-stimulated \([Ca^{2+}]_i\) oscillations and the sustained plateau induced by NMC (100 nM) in three of four cells (Fig. 6, C and D). In all cases, genistein symmetrically changed 340 and 380 nm fluorescences (Fig. 6, insets).

Because NMC increased \([LTC_4]_i\), the effects of the PLA\(_2\) inhibitor on Ca\(^{2+}\) spiking during NMC stimulation were examined and compared with that of BB. The \([Ca^{2+}]_i\) oscillation evoked by low doses of BB (0.1–10 nM) was not inhibited by ONO-RS-082 (10 µM) in 11 of 13 cells. The sustained \([Ca^{2+}]_i\) plateau elicited by BB (100 nM) was not significantly affected by ONO-RS-082 (10 µM) in four of four cells. In two of four cells, ONO-RS-082 (10 µM) inhibited \([Ca^{2+}]_i\) oscillations stimulated by NMC (0.3–10 nM) (Fig. 7A). The inhibitory effects of ONO-RS-082 on Ca\(^{2+}\) spiking were apparent with NMC (100 nM) stimulation. In three of three cells, ONO-RS-082 abolished the sustained \([Ca^{2+}]_i\) plateau (Fig. 7B). Note that after removal of ONO-RS-082 from the superfusion medium, a new and large \([Ca^{2+}]_i\) transient was observed in the presence of NMC. This was not observed with BB. Therefore, ONO-RS-082 was more potent in inhibiting Ca\(^{2+}\) spiking induced by NMC than that induced by BB. This is consistent with the data showing that NMC is 10–34 times more potent than BB in producing LTC\(_4\). In all cases, ONO-RS-082 symmetrically changed 340 and 380 nm fluorescences (Fig. 7, insets). These results indicate that U-73122 effectively inhibits the actions evoked by BB and NMC, whereas genistein and ONO-RS-082 effectively inhibit Ca\(^{2+}\) spiking evoked by NMC.

Effects of inhibitors of PLC, PTK, and PLA\(_2\) on amylase secretion induced by BB and NMC. U-73122 (5–7 µM) significantly reduced amylase secretion induced by high doses of BB (10–100 nM) (Fig. 8A). In contrast, U-73343 (10 µM), an inactive analog of U-73122, did not inhibit but enhanced amylase secretion stimulated by BB. Similarly, U-73122 (5 µM) significantly inhibited amylase secretion stimulated by NMC (1–100 nM) (Fig. 8B). U-73343 (10 µM), as a negative control, did not inhibit the NMC action. Genistein (100–300 µM) did not have a significant effect on amylase secretion stimulated by BB at any dose (0.1–100 nM), but it significantly inhibited the action of NMC (0.1–10 nM) (Fig. 9). Similarly, ONO-RS-082 (10–30 µM) did not alter amylase secretion induced by BB, but it significantly inhibited amylase secretion induced by both low (0.1 nM) and high (100 nM) concentrations of NMC (Fig. 10).

DISCUSSION

Erspamer et al. (10) first observed that crude methanol extracts of amphibian skin produced various actions on vascular smooth muscle cells, exocrine and endocrine secretion, and renal circulation and function.
This extract contained the tetradecapeptide BB (1). Subsequently, a structurally related GRP and NMC were isolated from the gastric tissue and spinal cord of pigs and other mammalian species (15, 22, 25, 30, 33). Currently, 13 BB-like peptides have been isolated from different amphibian species and grouped into three subfamilies according to their COOH-terminal tripeptide (9). BB and alytesin have the COOH-terminal His-Leu-Met-NH₂, the ranatensins and litorins have the COOH-terminal His-Phe-Met-NH₂, and the phyllolitorins have Ser-Leu (or Phe)-Met-NH₂. A shorter form of ranatensins, known as NMB, has also been isolated from the porcine spinal cord (24). GRP and NMC bind to the type 2 BB receptor, whereas NMB preferentially binds to the type 1 BB receptor (44). Note that an α-amidated COOH-terminal heptapeptide is conserved and shared by amphibian, avian, and mammalian BB-related peptides including BB, GRP, and NMC. Studies of the structural activities of smooth muscle preparations suggest that the COOH-terminal heptapeptide contains the minimal segment associated with biological activity and that the COOH-terminal nonapeptide of BB is as potent as the natural BB (5). Moreover, the α-amidated COOH-terminal methionine residue appears to be essential for the binding of BB and GRP to the high-affinity cell surface receptors and for the initiation of biological response (26).

It is well recognized that BB, GRP, and NMC cause amylase secretion through the BB type 2 receptor in mammalian pancreatic acinar cells (14, 31). Our study confirmed this observation in rat pancreatic acini; NMC was 30-fold more potent in stimulating amylase secretion than NMB. We also showed that in rat pancreatic acini, the α-amidated COOH-terminal heptapeptide BB-(8—14) or shorter peptides [e.g., BB-(9—14) and BB-(11—14)] did not evoke amylase secretion, indicating that the BB heptapeptide is not sufficient for biological activity. Because the decapeptide NMC-(5—14), BB-(1—14), and GRP-(1—27) were equipotent in stimulating amylase secretion, Gly²-Asp³-His⁷ (or Gln⁷) of the COOH-terminal decapeptide must be critical amino acids for full biological activity of the BB analogs. This possibility is supported by the observation that the heptapeptide of GRP [GRP-(21—27) or GRP-(8—14), see Fig. 1] had no effect on the binding of ¹²⁵I-GRP to the murine pancreatic membranes, whereas the octapeptide AcGRP [GRP-(7—14), see Fig. 1] completely inhibited ¹²⁵I-GRP binding (11). This indicates the importance of His⁷ for receptor binding.

In this study, we showed that the major difference between frog BB and mammalian NMC relates to their abilities to activate the PLA₂ pathway: NMC was 10–34 times more potent than BB in increasing intracellular [LTC₄], an index of AA production. The only difference between BB and NMC from the 5th to the 14th position is that the basic amino acid His⁷ in NMC is replaced by a nonionized (at the neutral pH), but polar, amino acid Gln⁷ in BB, which suggests that His⁷ is also a key amino acid for recognizing the PLA₂ pathway in mammalian pancreatic acinar cells. Note that only one amino acid difference has also been observed between the mouse BB receptors and Swiss 3T3 cell GRP receptors: Arg³⁰⁹ at the seventh transmembrane domain in mice is replaced by His³⁰⁹ in Swiss 3T3 cells (3, 34). It has been suggested that Asp⁷ in the second hydrophobic transmembrane domain may play an important role in ligand binding (34, 35). We predict that Asp, which is a negatively charged amino acid, may preferentially form an ionic bond with His⁷, a positively charged amino acid.
Previous studies have clearly demonstrated that activation of the BB receptor evokes phosphatidylinositol turnover by activating the G protein and PLC, resulting in the production of IP3 and DAG (16). IP3 stimulates Ca2+ release from intracellular stores, and DAG activates protein kinase C translocation from the cytosol to the plasma membrane (4, 27). Without exception, BB also increases IP3 and DAG levels to elicit intracellular Ca2+ release and PKC activation in pancreatic acini (21, 28). We confirmed these observations by showing that frog BB and porcine NMC equipotently increased IP3 concentration, which was inhibited by the PLC inhibitor U-73122. We also demonstrated that U-73122 inhibited Ca2+ spiking and amylase secretion elicited by BB and NMC. However, we found that higher concentrations of BB and NMC (10–100 nM) were required for sufficient production of IP3 and that the inhibitory actions of U-73122 on [Ca2+]i levels and amylase secretion were observed only if high doses of BB and NMC were used. This suggests that other intracellular signal transduction pathways may be involved in mediating amylase secretion stimulated by BB-like ligands.

Our study demonstrated that in rat pancreatic acinar cells, both BB and NMC stimulated PTK activity, which was measurable by RIA using RR-SRC peptide as a substrate. Previous studies using a specific antiphosphotyrosine antibody demonstrated that BB increased the phosphorylation of 120-, 115-, 90-, and 75-kDa proteins in Swiss 3T3 cells (6, 17, 50, 51). These are probably nonreceptor PTKs. BB has also been shown to stimulate oncogenes (e.g., c-myc and c-fos) via a PTK pathway in this cell line (16). Our study showed that the EC50 for NMC-activated PTK was 2 log units less than the EC50 for BB-activated PTK. Furthermore, the Ca2+ spiking and amylase secretion stimulated by NMC appear to be more sensitive to the PTK inhibitor genistein than the Ca2+ spiking and amylase secretion evoked by BB. This suggests that the BB receptor on rat pancreatic acini is probably coupled to the PTK pathway. Note that in contrast to the PLC pathway, which requires high doses of BB analogs for its activation, the PTK cascades appear to be activated by lower
concentrations of NMC. We and others (8, 18, 41) have reported that activation of the CCKA receptor in the rat pancreatic acini resulted in the tyrosyl phosphorylation of 105- and 85-kDa and pp60src proteins. Although the precise biological functions of these PTKs are still unknown, these nonreceptor PTKs may mediate extracellular Ca\(^{2+}\)-dependent pancreatic enzyme secretion (41).

Our study also demonstrated that the BB receptor in rat pancreatic acini is coupled to the PLA2-cascade. PLA2 is a Ca\(^{2+}\)-dependent esterase capable of catalyzing the hydrolysis of fatty-acid ester bonds at the sn-2 position of glycerophospholipid (42). Evidence suggests that the cytosolic 85-kDa PLA2, which produces AA by catalyzing phosphatidylcholine and phosphatidylethanolamine, plays a major role in intracellular signal transduction (2, 7, 42). AA produced via this cascade acts as a second messenger to release Ca\(^{2+}\) from intracellular stores in several cells, including pancreatic acinar cells (36, 37, 48). Recently, we demonstrated that the high-affinity CCK receptors are coupled to the PLA2-AA pathway to mediate Ca\(^{2+}\) oscillation and amylase secretion in rat pancreatic acini (37, 39). We also showed that a melittin-derived PLA2-activating protein elicits Ca\(^{2+}\) oscillation and amylase secretion accompanied by an increase in the intracellular AA metabolite HETE (38). Similar observations were made with activation of the BB receptors on rat pancreatic acini; both BB and NMC stimulated the production of the AA metabolite LTC\(_4\). These findings are similar to those reported in work with Swiss 3T3 cells, which showed that BB caused a rapid release of AA and prostaglandin E\(_2\) via the cyclooxygenase pathways, mediating a mitogenic response (23). These metabolic pathways of AA are different from those in pancreatic acini (i.e., lipooxygenase and cytochrome P-450 pathways) (38, 39). Note that NMC was more potent than BB in increasing [LTC\(_4\)] and that the PLA2 inhibitor ONO-RS-082 inhibited amylase secretion evoked by NMC but not by BB, suggesting that the BB receptor in rat pancreatic acini is also coupled to the PLA2 cascade. In contrast to the PLC pathway, the...
PLA₂ pathway may be activated by low and high doses of NMC, since the actions of NMC at 0.1 and 100 nM were inhibited by the PLA₂ inhibitor ONO-RS-082.

We conclude that in contrast to the nonmammalian BB receptor, which primarily uses the PLC pathway, the rat BB receptor is linked to three different signal transduction systems. Activation by the mammalian BB-like peptide NMC results in activation of the PLC, PTK, and PLA₂ pathways, evoking Ca²⁺ spiking and amylase secretion. It appears that His at the 7th position of BB analogs is a key amino acid to activate the PTK and PLA₂ pathways.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants R01-DK-32830 and SP 30-DK-34933.

Address for reprint requests: C. Owyang, 3912 Taubman Center, Univ. of Michigan, Ann Arbor, MI 48109-0362.

Received 4 November 1996; accepted in final form 7 December 1997.

REFERENCES


