Dual Pathways for Carbamylcholine-Stimulated Arachidonic Acid Release in Rat Pancreatic Acini

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Recent studies suggested the involvement of arachidonic acid in the mediation of pancreatic amylase release. However, an effect of carbamylcholine on arachidonic acid release has not yet been reported in the exocrine pancreas. This study was performed to evaluate the effect of carbamylcholine on arachidonic acid release and determine the underlying intracellular mechanisms. From enzymatic assays, phospholipase A2 and diacylglycerol lipase were activated by carbamylcholine and these activations were inhibited by the phospholipase A2 inhibitors, mepacrine and aristolochic acid, and by the diacylglycerol lipase inhibitor RHC 80267. Carbamylcholine also increased arachidonic acid release in a concentration-dependent manner. Both phospholipase A2 and diacylglycerol inhibitors partially inhibited carbamylcholine-stimulated arachidonic acid release. The phospholipase C inhibitor U73122 and the protein kinase C inhibitor staurosporine also caused partial inhibition. Arachidonic acid release by carbamylcholine was suppressed by the simultaneous addition of RHC 80267 with either phospholipase A2 inhibitors. Our data demonstrate that phospholipase A2 and diacylglycerol inhibitors partially inhibited carbamylcholine-stimulated arachidonic acid release. Both phospholipase A2 and diacylglycerol lipase were activated and arachidonic acid is released in pancreatic acini by carbamylcholine. Dual pathways are responsible for carbamylcholine-induced arachidonic acid release. One such pathway involves the sequential action of phospholipase C, protein kinase C and diacylglycerol lipase, whereas the other involves phospholipase A2 activation.

Introduction

The muscarinic agonist carbamylcholine (Cch) binds to its receptor and causes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2), and the production of inositol trisphosphate (IP3) and diacylglycerol (DAG). These two PIP2 metabolites are respectively recognized to mobilize intracellular calcium and to activate protein kinase C (PKC) (Berridge, 1984; Cook and Wakelam, 1991). Calcium mobilization by Cch is also associated to pancreatic enzyme secretion (Yule and Williams, 1992).

Among second messengers formed through the activation of multiple known effectors, arachidonic acid (AA) has been recognized as an important factor (Noar, 1991). In the exocrine pancreas, AA release in response to two cholecystokinin (CCK) analogs, cerulein (Dixon and Hokin, 1984) and CCK-octapeptide (Pandol et al., 1991), has been clearly demonstrated and its involvement in enzyme secretion has been postulated. AA can be generated via two major signaling pathways, one of which being phospholipase A2 (PLA2) and the other the PIP2 metabolite DAG, which on hydrolysis by DAG lipase, release AA (Irvine, 1982; Van den Bosch, 1980; Wang et al., 1994). In the exocrine pancreas, the source of AA has been identified from phospholipase C (PLC)-catalyzed breakdown of phosphatidylinositol followed by DAG lipase but not from PLA2 acting on phosphatidylinositol (Dixon and Hokin, 1984). Another source seems to involve a phospholipase A action on phosphatidylinositol (Pandol et al., 1991). Among other reported pathways, AA could be generated from the activation of phospholipase A1 followed by lysophospholipase B (Exton, 1990) or through the sequential activation of phospholipase D (PLD), phosphatidate phosphohydrolase (PPH) and di- and monoglyceride lipase (Martin and Wysolmerski, 1987). Although there are some reports describing AA release in response to cerulein (Dixon and Hokin, 1984) and CCK-octapeptide (Pandol et al., 1991) in the pancreas, and others on Cch-induced AA release from rat brain cortex membranes (Strosznajder and Samochcki, 1991), an effect of Cch on AA release from the exocrine pancreas has not been reported, nor any data on the intracellular signaling systems involved.

In the present studies, we, therefore, investigated the effect of Cch on AA release as well as the signal transduction pathways involved. These data indicate for the first time that Cch significantly activates PLA2 and DAG lipase activities and stimulates AA release in pancreatic acinar cells. Two pathways are described as responsible for such
Materials and Methods

Materials

Carbamylcholine, bovine serum albumin (BSA, Fraction V and BSA fatty acid-free), soybean trypsin inhibitor type 2-S (SBTI), N-2-hydroxyethylpiperezine-N'-2-ethanesulfonic acid (HEPES), standards for thin layer chromatography (TLC), propranolol, 5,8,11,14-eicosatetraynoic acid (ETYA), arachidonic acid (5,8,11,14-eicosatetraenoic acid), aristolochic acid, mepacrine, sucrose, ethylene diamine tetraacetic acid (EDTA), ethyleneglycol-bis-(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid (EGTA), leupeptin, pepstatin and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma (St. Louis, MO). Phosphatidylethanol (PEt) was from Avanti Polar Lipids (Birmingham, AL). Purified collagenase (1424 U/mg) was from Worthington Biochemicals (Freehold, NJ). RHC-80267 was a gift from Sandoz, Canada. Silica gel G TLC plates (28-200 mesh) were from Fisher (Pittsburgh, PA). [3H] myristic acid (56 Ci/mmol) and 1-stearyl-[3H]-myristic acid incorporation, HEPES-buffered solution as described above. For experiments without BSA was used.

Preparation of Pancreatic Acini

Pancreases from rats fasted overnight were removed and trimmed of fat and mesentery. A suspension of pancreatic acini was prepared as reported by Peikin (Peikin et al., 1978). Acini from five pancreaces were resuspended in 32 mL of an enriched HEPES-buffered solution ([in mM] 24.1 HEPES, 98 NaCl, 6 KCl, 2.1 KH2PO4, 0.5 CaCl2, 1.2 MgCl2, 5 sodium pyruvate, 5 sodium fumarate, 5 sodium glutamate, and 11.4 glucose, as well as 0.01% [w/v] SBTI, 2.5% [v/v] glutamine, 1% [v/v] essential vitamin mixture, and 1% [v/v] BSA, adjusted to pH 7.4). For experiments with [3H]-myristic acid incorporation, fatty acid-free BSA was used at concentration of 0.5% [w/v] in the same HEPES-buffered solution as described above. For experiments with [3H]-AA incorporation, HEPES-buffered solution without BSA was used.

Uptake of [3H]-AA into Pancreatic Acinar Cells

Acini from five pancreases were resuspended in 32 mL of HEPES-buffered solution and divided into flasks of 5 mL each. Acinar cells in each flask were incubated with [3H]-AA (5 μCi/mL) for 120 min at 37°C. At the start of the incubation period (1 min) and every 30 min thereafter, 1 mL of acini suspension was removed from each flask followed by a 30 s centrifugation to discard the supernatant. The AA incorporation was ended by the addition of 2 mL of 5% trichloroacetic acid (TCA) to the pellets. The mixture of the acinar cells and TCA was vortexed vigorously and centrifuged again. The radioactivity present in the supernatant and in the TCA-precipitated materials was then determined after addition of scintillation fluid and expressed as percent of the total radioactivity present in acini.

DAG Lipase Assay

Cell extracts were prepared as described by Cybulsky (1991). Acini were pretreated with DAG lipase inhibitor RHC 80267 at 150 μM in the appropriate groups for 15 min. A 15 min incubation followed in the presence or absence of 5 μM Cch. At the end of the incubation, acini were centrifuged and the pellet obtained was washed twice with an homogenization buffer containing 50 mM HEPES, 0.25M sucrose, 1 mM EDTA, 1 mM EGTA, 20 μM leupeptin, 20 μM pepstatin, 0.1 mM PMSF, and 0.01% SBTI (w/v), pH 7.4. Acinar cells were disrupted in a glass–glass homogenizer and the homogenate was centrifuged at 1000g for 10 min to separate membrane components. The supernatant was collected and proteins were determined by the method of Bradford (1976). The assay of DAG lipase was adapted from Prescott and Majerus (1982). The substrate mixture of 1-stearyl-2-[14C] arachidonoyl-glycerol and unlabeled DAG was dried under nitrogen and suspended in 0.05% Triton X-100. After sonication for 20 s, 5-μL aliquot of the substrate mixture was distributed into tubes to give final 200 μM concentration of DAG. The assay was initiated by adding the enzyme source containing 16 μg of protein and 7 mM CaCl2 and terminated by adding 2% sulfonic acid (HEPES), standards for thin layer chromatography plates. A solvent system consisting of hexane:diethyl ether:acetic acid (80:20:2) was used to separate AA which was identified as migrating with authentic standards detected using L2 vapor. Areas containing AA were scraped and radioactivity was determined in a liquid scintillation counter. Results are expressed as DPM/mg protein.

Phospholipase A2 Assay

PLA2 assay was performed according to the method described by Jelsema (1987). After they were incubated with 5 μM Cch for 15 min in the presence or absence of inhibitors, pancreatic acini were washed twice with the homogenization buffer containing 10 mM HEPES (pH 7.4), 1 mM EDTA, 1 mM EGTA, 0.34 M sucrose, 10 μg/mL leupeptin, 0.01% SBTI (w/v) and 1 mM PMSF. After homogenization in a glass–glass homogenizer, nuclei and...