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Dose-response for inhibition by amylin of cholecystokinin-stimulated secretion of amylase and lipase in rats

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Abstract

Background and aims: The neuroendocrine hormone amylin, cosecreted with insulin from pancreatic β-cells in response to nutrient ingestion, has several physiologic actions to limit the rate of nutrient uptake, including the slowing of gastric emptying.
Methods: To investigate whether amylin might modulate digestive enzyme secretion from the exocrine pancreas, anesthetized Sprague Dawley rats were cannulated via the pancreatic duct and the secretory response (flow, amylase and lipase) to cholecystokinin (1 μg s.c.) was measured in the absence and in the presence of 0.1, 0.3 and 1 μg s.c. doses of amylin.
Results: Amylin alone did not affect pancreatic secretion, but it dose-dependently inhibited cholecystokinin-stimulated amylase secretion by up to 58% and lipase secretion by up to 67%. The ED_{50}'s for these responses were 0.21 μg ± 0.18 log and 0.11 μg ± 0.05 log, respectively, doses that result in excursions of plasma amylin concentration that are within the reported physiological range. Amylin did not evoke cell signalling in the Ar42j model of pancreatic acinar cells, and responses to amylin were not observed in either Ar42j cells or isolated pancreatic acini in a microphysiometer indicating that the effect of amylin was indirect.
Conclusions: Inhibition of stimulated pancreatic enzyme secretion is likely to be a physiological, extrapancreatic, action of amylin. Amylinergic mechanisms modulating both gastric emptying and pancreatic enzyme secretion may thus match, respectively, the appearance of substrate and enzymes in the gut lumen.

Keywords: Pancreatic lipase secretion; Pancreatic amylase secretion; Nutrient intake; Pancreatitis; Ar42j cells; R_a

1. Introduction

Amylin, a 37 amino acid peptide hormone [1] cosecreted with insulin in response to nutrient stimuli from pancreatic β-cells [2] has several actions which have been identified from pharmacological blockade of endogenous peptide to be physiological. These include the inhibition of food intake [3–6], the inhibition of gastric emptying [7] and the inhibition of nutrient-stimulated glucagon secretion [8]. Collectively, these actions indicate a physiological role of amylin to restrict nutrient inflow into the blood [9,10], a gateway that is potentially as important as the subsequent disposal of nutrient in maintaining normal fuel balance.

Amylin-mediated effects to slow gastric emptying, apparent at concentrations within the physiological amylin range [7] is predicted to modulate the rate at which nutrient enters absorptive sections of the gut, and thereby, the demand for digestive enzyme. Since it is possible that related processes could be coordinated via a common pathway, our principal purpose in the current work was to investigate the possibility that amylin physiologically modulates secretion of pancreatic digestive enzymes.

In the present study in anesthetized rats, we measured the effect of different doses of amylin on CCK-stimulated secretion of lipase and amylase enzyme from the cannulated pancreas. We identify a potent, likely physiological, effect of amylin to inhibit stimulated pancreatic enzyme secretion, and discuss this discovery in terms of amylin's emerging role as a metabolic regulator. Results of this part of the study have been presented in a preliminary communication [11].

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2. Materials and methods

2.1. Materials

Rat amylin was produced in-house by solid-phase peptide synthesis (Lot AR905-80; AR518-92). Purity by reversed-phase HPLC was >98% and peptide content was 77%. Biological activity was confirmed by binding to amylin receptors in rat nucleus accumbens membranes [12]. Cholecystokinin octapeptide (CCK-8) was obtained from Peninsula Laboratories (Belmont, CA. Lots 034280 and 441021).

2.2. Animals

Male Harlan Sprague Dawley rats (334–399 g) were housed at 22.8 ± 0.8 °C in a 12:12 h light:dark cycle. All experiments were performed during the light cycle. Animals were fasted for approximately 20 h before experimentation but were given free access to water until the start of the experiment.

2.3. Surgery

Rats were anesthetized with 5% halothane, maintained with 2% halothane during surgery and with 1% halothane thereafter. Tracheotomy and cannulation of the right femoral artery were performed and body temperature was controlled with a thermoregulator (Model 73A, YSI, Yellow Springs, OH, USA) that switched a heated operating table. The femoral arterial line, used for blood sampling, was perfused with heparinized saline (2 U/mL) and connected to a pressure transducer for blood pressure recording (Spectramed P23XL transducer, Model 13-4615-58 amplifier, Gould, Cleveland, OH, USA). Through a midline incision two polyethylene cannulae were inserted into the common bile–pancreatic duct at a point about 0.5 cm above where the duct enters the pancreas. The first cannula was inserted up toward the liver to collect bile. The other end of this cannula was placed into the duodenum through a small incision in the duodenum. Thus, bile flowed directly from the liver to the small intestine, being shunted away from the pancreas completely. A second polyethylene cannula inserted into the common bile–pancreatic duct near the first was directed toward the pancreas to collect pancreatic juice. The pancreatic duct was ligated at its entry into the duodenum, forcing secreted pancreatic juice into the collection cannula.

2.4. Treatment groups

Saline, rat amylin, CCK-8, or both peptides were injected subcutaneously at $t=0$ min. Saline only ($n=5$); Amylin only, 1 μg ($n=5$), amylin only 10 μg ($n=5$); CCK-8 only 1 μg ($n=6$); CCK-8 1 μg in combination with rat amylin (0.1, 0.3, 1 μg; $n=6$, 7, 6 respectively).

2.5. Enzyme assays

Pancreatic juice was collected over 15 min intervals between $t=-30$ to +90 min. The volume of pancreatic juice (measured by weight) and activities of amylase and lipase (secreted in an active form) were determined for each 15-min aliquot (Lipase-PS assay kit #805-A; Amylase 3 assay kit #577-3, Sigma Diagnostics, St. Louis, MO). Pancreatic juice was diluted 1:2000 before assay. Enzyme secretion was expressed in units per 15 min obtained by multiplying activity by volume collected. One unit of amylase activity is defined as that required to liberate 1 mg of maltose from starch in 3 min at pH 6.9 at 20 °C. There are ~700–1400 U/mg protein. One unit of lipase activity is defined as that required to hydrolyze 1 μEq of fatty acid from a triglyceride in 1 h at pH 7.7 at 37 °C. There are ~20,000–100,000 U/mg protein.

2.6. Effects of amylin on inositol phosphate production in Ar42j cells

Ar42j cells, a model of pancreatic acinar cells derived from a pancreatic carcinoma line, exhibit many aspects of acinar behavior, including secretion of amylase in response to stimulation with pituitary adenylate cyclase activating peptide (PACAP38) [13–15]. PACAP38-mediated signalling in acinar and Ar42j cells appeared to be via other than cAMP [13], and is now recognized as being via activation of phospholipase C and mobilization of intracellular calcium [16]. Using the response to PACAP27 (Lot ZK266, Bachem, Torrance, CA) and PACAP38 (Lot ZM766, Bachem) as a positive control (indication that cells and signaling pathways were intact), effects of amylin on phospholipase C activation were tested in Ar42j cells. Ar42j cells (ATCC, Rockville, MD) were cultured in Ham's F12K medium containing 20% fetal bovine serum before being distributed into 12 well plates to grow until confluent. After overnight incubation with 2.2 μCi/mL [2-^{3}H]-*myo*-inositol (ICN, Costa Mesa CA), cells were rinsed and incubated with phosphate-buffered saline containing 0.1% bovine serum albumin and 5.5 mM glucose for 20 min, and then incubated for 10 min in the presence of 20 mM LiCl. Peptides were then added to the cells as indicated and incubated for an additional 20 min. Reactions were terminated by the addition of 1 mL methanol:2 M HCl (9:1). Inositol phosphates were extracted as described previously [17] and results expressed as DPM of inositol monophosphate produced/well and are triplicate determinations from a representative experiment.

2.7. Effects of amylin on responses in isolated acini and in Ar42j cells in a microphysiometer

Rates of acid production, as measured with a Cytosensor microphysiometer (Molecular Devices, Menlo Park, CA),