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Role of endogenous amylin in glucagon secretion and gastric emptying in rats demonstrated with the selective antagonist, AC187

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Abstract

Amylin is a 37-amino acid polypeptide co-secreted with insulin from the pancreatic β-cells. It complements insulin's stimulation of the rate of glucose disappearance (R_d) by slowing the rate of glucose appearance (R_a) through several mechanisms, including an inhibition of mealtime glucagon secretion and a slowing of gastric emptying. To determine if endogenous amylin tonically inhibits these processes, we studied the effects of the amylin receptor blocker AC187 upon glucagon secretion during euglycemic, hyperinsulinemic clamps in Sprague–Dawley® (HSD) rats, upon gastric emptying in HSD rats, and upon gastric emptying and plasma glucose profile in hyperamylinemic, and genetically obese, Lister Albany/NIH rats during a glucose challenge. Amylin blockade increased glucagon concentration, accelerated gastric emptying of liquids, and resulted in an exaggerated post-challenge glycemia. These data collectively indicate a physiologic role for amylin in glucose homeostasis via mechanisms that include regulation of glucagon secretion and gastric emptying.

Keywords: AC187; Glucagon; Gastric emptying

1. Introduction

Amylin [1] is a 37-amino acid peptide that is co-localized with insulin in pancreatic β-cells and co-secreted with insulin [2] in response to nutrient stimuli. Complementing insulin's effect to increase the rate of glucose disappearance, R_d, amylin slows the rate of glucose appearance, R_a, via several mechanisms [3]. These include slowing the rate of gastric emptying (thereby the rate of presentation of nutrients to absorptive bowel) and inhibiting glucagon secretion (and hence, endogenous glucose production). Each of these important determinants of normal postprandial glucose flux has been shown to be potently regulated by amylin (or amylin analogs) in rodents and humans [3].

Molecular characterization of amylin receptors [4,5] did not occur until 13 years after identification of the ligand, despite earlier identification of high affinity binding sites [6]. This circumstance, and a low potency for certain amylin actions (some of which were ultimately attributable to cross-reaction at calcitonin gene-related peptide [CGRP] receptors), prompted some commentators to question the physiologic relevance of amylin. The present work examines whether amylin's effects on glucagon secretion and gastric emptying are physiologic (evoked by endogenous amylin).

Several lines of evidence support the proposal that amylin's slowing of gastric emptying is physiologic. The EC50 for this effect of amylin in rats approached reported endogenous concentrations [7]. An accelerated rate of emptying in amylin-deficient BioBreeding (BB) rats was consistent with loss of an endogenous tonic restraint on gastric emptying [7]. Although the physiochemical properties of human amylin preclude its clinical use, the human amylin analog, pramlintide, has similar potency and enables human experimentation with amylin agonists. Studies in patients with type 1 and insulin-treated type 2 diabetes have shown that pramlintide slows the rate of gastric emptying [8,9] at doses that result in near-physiologic plasma amylin concentration.

There are similar indications supporting a physiologic glucagonostatic effect. Amylin infusions dose-dependently inhibited arginine-stimulated glucagon secretion in rats with an EC50 that approached endogenous amylin concentrations [10].

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In studies with patients with diabetes, exaggerated postprandial glucagon profiles were ameliorated by pramlintide delivered at doses resulting in physiologic amylin concentration [11,12].

However, it is often difficult to determine from studies using exogenously administered peptide whether an effect of endogenous peptide is present. In those cases, a more secure inference of an effect of endogenous ligand can be obtained when its activity is blocked with sufficient doses of a selective antagonist.

The Kd of amylin for its receptors was 27 pM [13]. The Ki of AC187 in the same preparation can be estimated from the equation $Ki = IC50/(1+[L]/Kd)$ to be 275 pM (IC50 480 pM, $[L]=20$ pM) [14]. AC187 is comparatively selective for amylin receptors, displacing amylin from them with 400× the potency with which it displaces radiolabelled CGRP from its receptors and with 38× the potency with which it displaces calcitonin from its receptors [15]. AC187 and other amylin antagonists have been used previously to identify effects of endogenous amylin on insulin secretion, muscle lactate production, and appetite control.

In the current experiments, the activity of endogenous amylin was blocked with AC187. The resulting effects on gastric emptying, glucagon secretion, and control of glucose profiles after an oral nutrient challenge suggest that amylin controls these processes physiologically. These data have been communicated previously in abstract form [16–20].

2. Materials and methods

2.1. Animals

Three separate sets of experiments were conducted to determine (a) if endogenous amylin tonically inhibits glucagon secretion (Experiment 1); (b) if endogenous amylin tonically inhibits gastric emptying (Experiment 2), and (c) if endogenous amylin affects gastric emptying and plasma glucose concentration post-gavage in genetically obese Lister Albany/NIH (LA/N-cp) rats (Experiment 3).

Sixty-one male HSD rats (Harlan, Indianapolis, IN) and 11 male LA/N-cp rats (OL Tulp, Drexel University, Philadelphia, PA), previously shown to be hyperamylinemic [21], were studied. All animals were housed at 22±0.8 °C in a 12:12 h light/dark cycle, and fed Diet LM-0485 (Teklad, Madison, WI) and water *ad libitum*. All experiments were performed during the light cycle and were in accordance with institutional guidelines for animal care and use.

2.2. Surgical procedures

2.2.1. Experiment 1

Thirty-eight male HSD rats (age: 91±2 days, weight: 359±3 g) were fasted for 20 to 24 h before surgery. Anesthesia was induced with 5% halothane, and maintained at 0.7% to 2.0% during surgery, and thereafter at 0.7% to 1.0%. Body temperature was feedback controlled using a heated operating table. The animals were cannulated via the right femoral artery for blood sampling and pressure monitoring (Spectramed P23XL transducer, Model 13-4615-58 amplifier, Gould, Cleveland, OH). A primed/continuous infusion (120 mU/kg/h) of 12 mU insulin and a variable infusion of 20% D-glucose via the saphenous vein were employed to maintain plasma glucose concentration measured at 10 min intervals at approximately 5.8 mM for 60 min. The animals received an IV infusion of either 3 mg AC187 (Amylin Pharmaceuticals, Inc., San Diego, CA) in 0.5 mL saline/h ($n=8$) or 0.9% saline ($n=30$) for the duration of the clamp. Arterial blood samples were collected at baseline and at 30, 45, and 60 min for measurement of glucagon.

2.2.2. Experiment 2

Twenty-three conscious, non-fasted HSD rats (age: 77–101 days, weight: 378±4 g) were studied to measure the effects of endogenous amylin blockade on gastric emptying. Rats were first gently restrained and the tip of the tail was anesthetized with Anthocaine (2% lidocaine hydrochloride, Anpro Pharmaceutical, Arcadia, CA) for blood sampling. At 5 min prior to gavage, rats received an intravenous bolus injection of either 3 mg AC187 in 0.1 mL saline ($n=13$) or saline alone ($n=10$). Rats were gavaged (time=0 min) with 5 μCi [3−^{3}H] glucose (lot 3165-036, Dupont, Boston, MA) in 1 mL sterile water. Blood samples were collected from the tail vein at −15, 0, 5, 15, 30, 60, and 90 min.

2.2.3. Experiment 3

Eleven obese, LA/N-cp rats (age: 208±18 days, weight: 660±30 g), fasted 24 h, were studied in a crossover design on two occasions separated by 14 days. On each occasion, they were injected subcutaneously with either 3 mg AC187 in 0.1 mL saline, or with saline alone, immediately prior to gavage with 1 mL 50% glucose containing 50 μCi of [3C–^{3}H] glucose. Blood samples were collected from lidocaine-anaesthetized tail veins at 0, 15, 30, 60, 90, and 120 min for plasma glucose cocentrations and for [3−^{3}H] glucose appearance (to measure gastric emptying of liquids) and processed as described above in Experiments 1 and 2.

2.3. Amylin receptor blocker AC187

Endogenous amylin action was blocked in all three sets of experiments with the amylin receptor antagonist AC187 [15]. AC187 is a 25-amino acid peptide (*N*-acetyl-Val-Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Asn-Thr-Tyr-NH_2). AC187 binds to amylin receptors in rat *nucleus accumbens* with high (79 pM) affinity, while having much lower affinity for CGRP and calcitonin binding sites [6]. Receptor blockade with 3 mg AC187 is sufficient to prevent responses to even supraphysiologic doses of amylin in vivo [22,23].

2.4. Assays

In Experiments 1 and 3, plasma glucose concentration was measured using an immobilized enzyme chemistry glucose/lactate analyzer (model 2300-STAT; Yellow Springs Instruments). Glucagon was assayed by radioimmunoassay (kit 07-152101, ICN Biochemicals, Costa Mesa, CA) following collection of 100 μL plasma into tubes containing 1 μL