Journal of Cellular Biochemistry 55S:12–18 (1994)

Amylin Regulation of Fuel Metabolism

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Abstract The 37-amino acid amylin, co-secreted from the pancreatic β cells with insulin in response to nutrient stimuli has actions in a number of tissues of metabolic interest. In muscle it opposes glycogen synthesis and activates glycogenolysis, an action likely to underly its stimulation of lactate flux. Amylin therefore appears to have the effect of transposing carbon from peripheral stores to the liver, where it is made available for hepatic synthesis of glucose, glycogen, and lipid. While amylin induces insulin resistance in skeletal muscle, it does not oppose insulin action in fat and may therefore favor fuel deposition in this tissue. Amylin acts on the β cell to inhibit insulin secretion. Relative impairment of insulin secretion, muscle insulin resistance, relatively preserved insulin sensitivity in fat, increased lactate turnover, and increased hepatic glucose production are features of insulin resistance and early non-insulin-dependent diabetes mellitus. Amylin is elevated in these dysfunctional metabolic states and may be involved in their pathogenesis.

Key words: skeletal muscle, insulin resistance, muscle glycogen, lactate, glucose, liver, pancreatic β cells

INTRODUCTION

Amylin, a 37-amino acid peptide from pancreatic β cells [1], has actions in a number of tissues. Some of the actions of amylin, for example, a decrease in plasma calcium and vasodilation, may be attributable to activation of receptors for related peptides such as calcitonin or calcitonin gene-related peptide (CGRP), to which amylin is related [2]. Since this paper focuses on amylin's role in the regulation of fuel metabolism, its actions in the main tissues of metabolic interest, tissues where fuel storage and conversion occurs (i.e., muscle, fat, and liver), are considered. Because of its role in generating the key endocrine signals to which these tissues respond, this paper also considers actions at the endocrine pancreas. These separate actions are then inserted into an integrated picture of how amylin may act in the intact organism, with a commentary on the role it may play in the disposition of metabolic fuels. Several of the actions of amylin were initially defined at supraphysiological concentrations. Biological actions evoked at concentrations of amylin reported in vivo are described as well.

Received October 19, 1993; accepted January 24, 1994.

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MUSCLE GLYCOGEN

The first biological action of amylin to be identified by Leighton and Cooper, in 1987 [3], was in skeletal muscle. In the insulin-stimulated isolated rat soleus muscle, amylin dose-dependently inhibited the incorporation of radioglucose into glycogen. Interest in this finding followed the recent recognition of the dominant role of muscle glycogen metabolism in determining insulin sensitivity/resistance [4]. Amylin concentrations measured by immunoassay or by radioreceptor assay in incubation medium comparable to concentrations measured by immunoassay in plasma evoke such responses in the isolated soleus muscle [5]. When measured by radioreceptor assay, the EC_{50} for the actions of human amylin on rat muscle was 220 pM. The nature of the interaction between insulin and amylin in muscle was subsequently found to be noncompetitive [5]. That is, amylin diminished the muscle glycogen response to insulin at each and every concentration of insulin. Responses to high insulin levels, as are found in insulin-resistant individuals, were just as susceptible to amylin as were responses at low insulin concentrations. Another provocative outcome of this analysis was that the response to an increasing strength of a fixed-ratio mixture of insulin and amylin (as has been proposed by some authorities to exit from the β cell) is bell shaped [5]. Since there are many examples of cosecretion in

neuroendocrine systems, we may wonder how common similar relationships are in endocrine control systems (Fig. 1).

Amylin's effects on muscle glycogen have been attributed both to inhibition of glycogen synthase [6] and to activation of glycogen phosphorylase [6,7], the rate-limiting enzymes in the synthesis and breakdown of glycogen. Amylin's predominant action to mobilize muscle glycogen is difficult to distinguish from an effect to inhibit glycogen synthesis when labeled glucose is followed into subsequently extracted glycogen. However, this action can be observed following the [3-^{3}H]glucose prelabeling of glycogen. The advantage of labeling glucose with tritium on the 3-position is that it is retained during glycogen formation and breakdown but is lost to water during the irreversible conversion of hexose to triose in glycolysis. The appearance of tritiated water is therefore an indicator of glycolysis of glucose thus labeled [4]. When the muscle glycogen pool was prelabeled with [3-^{3}H]glucose by first exercising rats and then infusing them with insulin and labeled glucose, the generation of tritiated water could then be used to indicate the metabolism of moieties that originated in glycogen. The rate of tritiated water generation increased severalfold after administration of amylin, indicating amylin's effect in mobilizing glycogen stores in vivo [8].

GLUCOSE TRANSPORT

Because amylin inhibits uptake of glucose into peripheral tissues [9–12] during euglycemic clamp procedures, the question arises as to whether it depresses glucose transport in muscle. In vitro studies in glucose transport have reported contradictory findings [11,13–15]. Since the observed effects on glucose uptake could reflect actions at glycogen synthase and phosphorylase, this issue remains unresolved.

HYPERLACTEMIA

One consequence of muscle glycogenolysis is release of lactate. Indeed, plasma lactate rapidly rises following amylin administration, in a manner analogous to that described by Cori and Cori following the administration of another glycogenolytic hormone, adrenaline (epinephrine) [16].

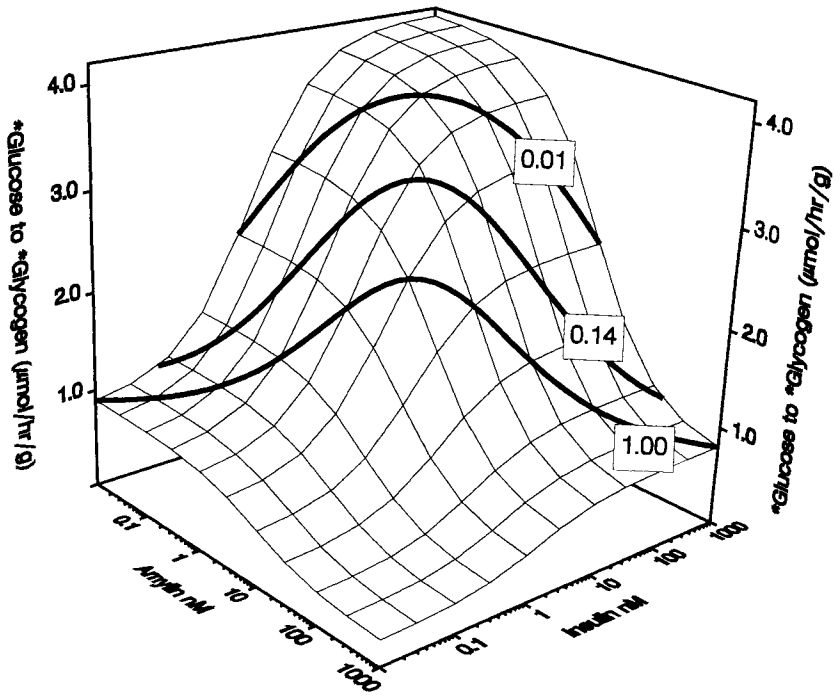

Fig. 1. Concentration–response surface for the effects of amylin and insulin upon glycogen metabolism in rat soleus muscle. A family of insulin responses is observed when viewed from the insulin axis, and a family of amylin responses is observed from the amylin axis. Heavy lines define the bell-shaped responses obtained when amylin and insulin are present in the fixed ratios indicated. Preparation is the isolated stripped soleus muscle, incubated in Krebs–Ringer bicarbonate buffer. Response is the rate of incorporation of [U-^{14}C]glucose over 1 hr into subsequently extracted glycogen.

Moreover, we have found that lactate release from isolated skeletal muscle is increased by amylin [Lupien et al., unpublished observations]. Significant increases in plasma lactate in rats have been reported with amylin doses calculated to increase plasma amylin by <150 pM [17], levels that have been reported for insulin resistant rodents [18,19]. Further evidence that the lactemic response occurs at endogenous concentrations of amylin is presented in studies using amylin antagonists (see section titled Acute Effects of Endogenous Amylin).

HYPERGLYCEMIA

Several groups have shown that amylin increases endogenous glucose production in rats, as measured by tritiated tracer dilution. This occurred during hyperinsulinemic euglycemic clamp procedures [9,10,12] as well as under nonclamped conditions during amylin infusions [20], in the latter case associated with an increase in plasma glucose concentration. Amylin-induced increases in plasma glucose are dose dependent [17]. A dose-dependent increase in plasma glucose following subcutaneous injections of the amylin agonist AC137 (^{23,28,29}Pro human amylin) has been seen in human volunteers (Moyses and Kolterman, in preparation).

In the fasted rat, where gluconeogenesis is enhanced and liver glycogen is depleted, amylin was both more effective and more potent than glucagon in elevating plasma glucose [21]. It is clear that the mechanism of hyperglycemia is distinct from that of glucagon [22]. While glucagon acts by breaking down liver glycogen, amylin's hyperglycemic effects appear to result, at least in part, from enhanced gluconeogenesis. For example, the increase in plasma lactate precedes the increase in plasma glucose following amylin administration. Further, in the fasted state, when gluconeogenesis is activated, the increase in glucose after amylin is greater than in the fed state and the increase in lactate is smaller, consistent with a greater draw-off of lactate and conversion to glucose [22]. Indeed, in further support of the idea that amylin's hyperglycemic effect is mediated at least partly through increased lactate supply, we have shown that increases in plasma lactate comparable to those evoked by amylin administration, but caused by infusions of lactate, resulted in comparable increases in glucose [23].

Impaired peripheral glucose disposal, as seen in euglycemic clamp studies [9–12], may also contribute to amylin-evoked hyperglycemia. Amylin has been reported to modulate insulin-sensitive processes such as glycogen synthesis. It could also impair glucose-stimulated glucose disposal through its glycogenolytic action by flooding the cell with glucose and reducing the gradient down which glucose transport occurs (Fig. 2).

DIRECT EFFECTS IN LIVER

Reports are contradictory as to whether amylin has direct effects on hepatocytes. In addressing this question, we have developed the "hyperlactemic clamp" in anesthetized rats where plasma lactate is maintained at a steady level by an infusion of sodium lactate varied in response to frequent lactate measurements. By clamping lactate, direct effects of amylin independent of those evoked by varying lactate concentration may be observed. During hyperlactemic clamping, endogenous glucose production (measured by tracer dilution) and plasma glucose levels rose. In further experiments in anesthetized rats, the lactate clamp was combined with a hyperglycemic clamp. Acute pancreatectomy was performed to eliminate the effects of endogenous pancreatic hormones, including insulin, glucagon, and endogenous amylin. Insulin was infused at a low dose to maintain a basal level of glucose utilization. During the 60 min following a 100-μg subcutaneous injection of rat amylin, endogenous glucose production increased by 51%. These data appear to indicate a direct effect of amylin on the liver to increase glucose

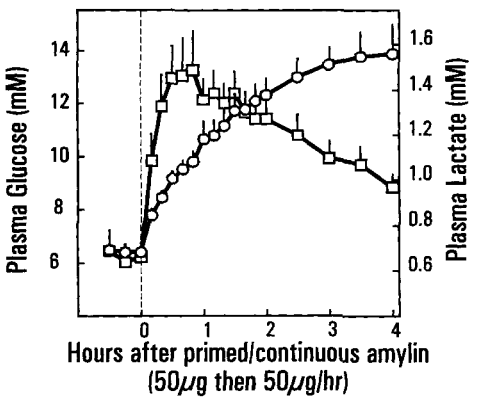

Fig. 2. Changes in plasma lactate (□) and glucose (○) following primed/continuous infusion of amylin into fasted anesthetized rats (n = 8).

production. However, the release of glucose from other tissues, such as muscle [8] cannot be excluded as a mechanism to explain the observed increase in endogenous glucose production.

CORI CYCLE

To summarize thus far, it appears that, in rats at least, amylin can shift carbon from muscle glycogen to the liver. That is, it activates the return limb of the Cori cycle [24]. The relevance of the Cori cycle has been enhanced by the discovery that in both rats [25,26] and humans, a substantial portion, if not the majority, of liver glycogen appears to be regenerated postprandially via the "indirect," gluconeogenic pathway (to which the Cori cycle contributes), rather than directly from glucose (the "direct" pathway) (Fig. 3).

FAT

To date, amylin has not been observed to have any effects in adipocytes, in regard to lipogenesis [27], lipolysis, or antilipolysis [28]. Does this mean that amylin cannot play a role in fat metabolism? In fact, it appears that the converse may be the case. While amylin produces insulin resistance in muscle, it does not do so in fat. The effects of the compensatory hyperinsulinemia associated with peripheral (mainly muscle) insulin resistance would then be most manifest in fat. Furthermore, in mobilizing lactate from the periphery, amylin can increase the hepatic supply of its preferred lipogenic substrate. So by several mechanisms, it is believed that amylin will favor fuel storage in fat over that in muscle.

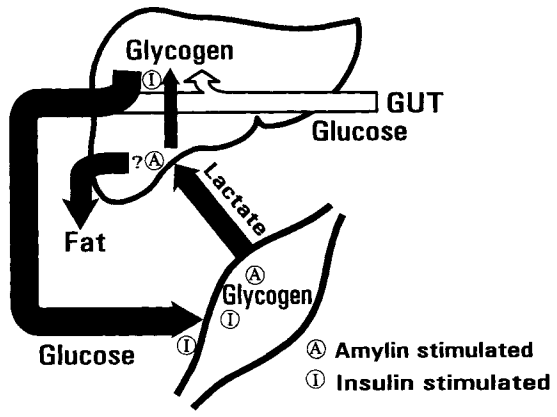

Fig. 3. Proposed role of amylin as a partner to insulin in stimulating Cori cycle activity and liver glycogen synthesis by the "indirect" pathway. Insulin activates peripheral uptake of glucose, largely into muscle glycogen following a glucose load. Amylin activates glycogenolysis and release of peripheral lactate for synthesis into other fuel stores at the liver. A direct effect of amylin at the liver, independent of its effect at peripheral tissues is possible.

PANCREAS

The weight of available evidence now supports the conclusion that amylin acts to inhibit insulin secretion. This has been shown in a variety of preparations, ranging from the whole animal [29], to the isolated perfused pancreas [30], to the isolated islet [31], to the isolated β cell [32,33]. Indicative of endogenous amylin's ability to inhibit insulin release are experiments where amylin antagonists [34,35] enhanced insulin secretory responses (Fig. 4).

CHRONIC EFFECTS OF ENDOGENOUS AMYLIN

But can a role for endogenously secreted amylin be demonstrated outside of the pancreas? Our first approach at this question was similar to one employed by Mahler et al. > 20 years ago [36,37]. Insulin-resistant rodents, in our case Fatty Zucker rats that overexpress insulin and amylin 5- to 10-fold, were administered streptozotocin (STZ) to ablate their β cells and were then treated with insulin implants to restore

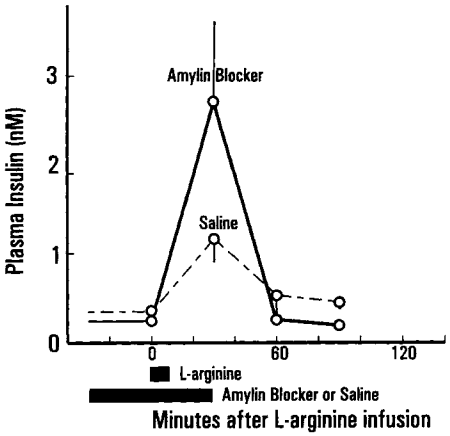

Fig. 4. Enhancement of the arginine-stimulated insulin response in rats preinfused with amylin antagonist. Following administration of [8–37] human CGRP, which blocks both amylin and CGRP receptors, there was an enhancement of the insulin response to a given L-arginine load. A similar response was observed following infusion of [8–32] salmon calcitonin, which blocks amylin, but not CGRP. These data support an autocrine role for endogenously secreted amylin, in limiting further β-cell secretion.

their previous hyperinsulinemia. After 6 weeks of such treatment (during which time amylin levels had presumably been reduced due to β-cell ablation), we found a marked improvement in insulin response measured in the isolated soleus muscle assay (that is, a reduction of insulin resistance [38]). Similarly, reduction of amylin activity by chronic administration of amylin antagonists increased insulin sensitivity in soleus muscles of both normal and Fatty Zucker rats (Young et al., unpublished observations). These data suggest a chronic effect of amylin to modulate insulin sensitivity of muscle glycogen metabolism.

ACUTE EFFECTS OF ENDOGENOUS AMYLIN

Is there an acute effect of endogenous amylin beyond the pancreas? In a recent series of experiments, the lactate surge in response to an intravenous glucose challenge was observed in the presence of matched changes in plasma glucose and insulin. The mechanism and tissue source of lactate released postprandially has been the cause of considerable debate. Greater glycolytic flux stimulated by increases in both glucose and insulin has been held to constitute the drive for this increased lactate production. Candidate tissues in which this might occur have included not only the insulin-sensitive tissues, muscle and fat, but also tissues in which intracellular glucose concentrations approach or exceed those in the interstitium, such as liver and gut. We carefully quantified the postglucose lactate surge in anesthetized rats in the presence or absence of the selective amylin antagonist AC187. When AC187 was present, the lactate surge was suppressed by ~50%, a finding consistent with the presence of an amylin-mediated component to the postprandial release of lactate in normal animals [36]. It appears that to account for postprandial lactate production, we should now consider amylin-stimulated processes and amylin-sensitive tissues, including glycogenolysis in skeletal muscle (Fig. 5).

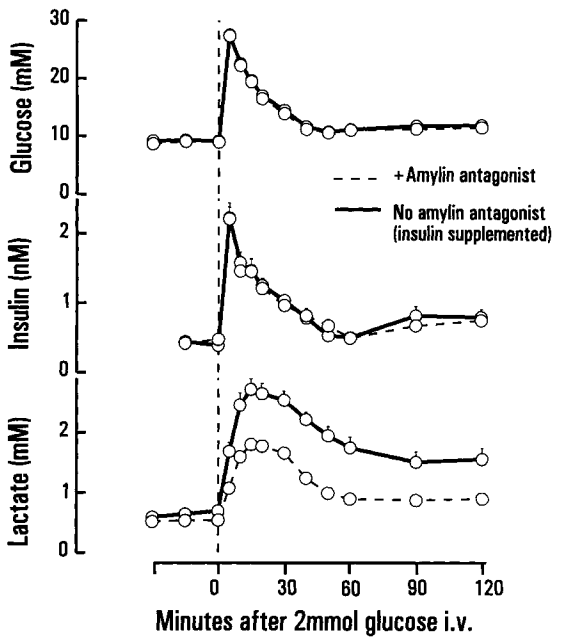

Fig. 5. Lactate surge in anesthetized rats following an intravenous glucose challenge, with and without preinfusion of the specific amylin blocker, AC187. Since the insulin response in the AC187-treated group was enhanced for the reasons indicated in Figure 4, insulin responses were matched by a s.c. insulin supplement in the group not treated with AC187. In spite of identical glucose and insulin profiles, the lactate profile in rats given the amylin blocker was much reduced, indicating that in addition to glucose and insulin-driven processes, postprandial lactate is also partly determined by a process sensitive to endogenously secreted amylin.

SUMMARY

A considerable body of evidence supports the view that amylin can regulate the transposition of carbon from muscle, via lactate, to liver, where it is available for synthesis into glucose, glycogen and fat. This role may be envisaged as being complementary to that of insulin, which is responsible for the initial deposition of dietary carbohydrate into muscle. In addition to its role as a metabolic regulator in the periphery, amylin apparently also modulates the metabolic signal represented by insulin. These actions point to a role in normal fuel homeostasis. Further evidence also supports the idea that amylin deficit or excess contributes to metabolic dysfunction in diabetes and other common disorders of carbohydrate and fat metabolism [1].

ACKNOWLEDGMENTS

I gratefully acknowledge the many workers from Amylin Pharmaceuticals, many of whose names are cited below, for their diligence in constructing the knowledge base to which this article relates. I also thank Nancy Gallaher-Wakefield for secretarial assistance.

REFERENCES

1. Rink TJ, Beaumont K, Koda J, Young A (1993): Structure and biology of amylin. Trend Pharm Sci 14:113–118.

2. Cooper GJS, Leighton B, Willis AC, Day AJ (1989): The amylin superfamily: A novel grouping of biologically active peptides related to the insulin A-chain. Prog Growth Factor Res 1:99–105.
3. Leighton B, Cooper GJS (1988): Pancreatic amylin and calcitonin gene-related peptide cause resistance to insulin in skeletal muscle in vitro. Nature 335:632–635.
4. Young AA, Bogardus C, Wolfe-Lopez D, Mott DM (1988): Muscle glycogen synthesis and disposition of infused glucose in humans with reduced rates of insulin-mediated carbohydrate storage. Diabetes 37:303–308.
5. Young AA, Gedulin B, Wolfe-Lopez D, Greene HE, Rink TJ, Cooper GJS (1992): Amylin and insulin in rat soleus muscle: Dose responses for cosecreted noncompetitive antagonists. Am J Physiol 263(2 Pt 1):E274–81.
6. Deems RO, Deacon RW, Young DA (1991): Amylin activates glycogen phosphorylase and inactivates glycogen synthase via a cAMP-independent mechanism. Biochem Biophys Res Commun 174:716–720.
7. Young AA, Mott DM, Stone K, Cooper GJS (1991): Amylin activates glycogen phosphorylase in the isolated soleus muscle of the rat. FEBS Lett 281:149–151.
8. Young A, Carlo P, Smith P, Wolfe-Lopez D, Pittner R, Wang M, Rink T (1993): Evidence for release of free glucose from muscle during amylin-induced glycogenolysis in rats. FEBS Lett 334:317–321.
9. Molina JM, Cooper GJS, Leighton B, Olefsky JM (1990): Induction of insulin resistance in vivo by amylin and calcitonin gene-related peptide. Diabetes 39:260–265.
10. Koopmans SJ, vanMansfeld ADM, Jansz HS, Krans HMJ, Radder JK, Frölich M, deBoer SF, Kreutter DK, Andrews GC, Maassen JA (1991): Amylin-induced in vivo insulin resistance in conscious rats: The liver is more sensitive to amylin than peripheral tissues. Diabetologia 34:218–224.
11. Young DA, Deems RO, Deacon RW, Mcintosh RH, Foley JE (1990): Effects of amylin on glucose metabolism and glycogenolysis in vivo and in vitro. Am J Physiol 259(3 Pt 1):E457–E461.
12. Betts JJ, Lupien JR, Horton ES (1991): Amylin induces in vivo insulin resistance in rats. Diabetes 40(Suppl 1):35A.
13. Zierath JR, Galuska D, Engstrom A, Johnson KH, Betsholtz C, Westermark P, Wallberg-Henriksson H (1992): Human islet amyloid polypeptide at pharmacological levels inhibits insulin and phorbol ester-stimulated glucose transport in in vitro incubated human muscle strips. Diabetologia 35:26–31.
14. Kreutter D, Orena SJ, Andrews KM (1989): Suppression of insulin-stimulated glucose transport in L6 myocytes by calcitonin gene-related peptide. Biochem Biophys Res Commun 164:461–467.
15. Leighton B, Foot E (1990): The effects of amylin on carbohydrate metabolism in skeletal muscle in vitro and in vivo. Biochem J 269:19–23.
16. Cori C, Cori GT (1929): The mechanism of epinephrine action. IV. The influence of epinephrine on lactic acid production and blood sugar utilization. J Biol Chem 84:683.
17. Young AA, Rink TJ, Wang MW (1993): Dose response characteristics for the hyperglycemic, hyperlactemic, hypotensive and hypocalcemic actions of amylin and calcitonin gene-related peptide-I (CGRPα) in the fasted, anaesthetized rat. Life Sci 52(21):1717–1726.
18. Gill AM, Yen TT (1991): Effects of ciglitazone on endogenous plasma islet amyloid polypeptide and insulin sensitivity in obese-diabetic viable yellow mice. Life Sci 48:703–710.
19. Jamal H, Bretherton-Watt D, Suda K, Wang ZL, Ghatei MA, Williams S, Bloom SR (1991): Changes in islet peptide content after dexamethasone treatment. J Endocrinol 129(Suppl):93.
20. Young AA, Wang MW, Rink TJ, Cooper GJS (1991): Effects of intravenous injections of amylin in the fasted, anaesthetized rat. J Physiol (Lond) 438:250P.
21. Wang MW, Carlo P, Rink TJ, Young AA (1991): Amylin is more potent and more effective than glucagon in raising plasma glucose concentration in fasted, anesthetized rats. Biochem Biophys Res Commun 181:1288–1293.
22. Young AA, Cooper GJS, Carlo P, Rink TJ, Wang MW (1993): Response to intravenous injections of amylin and glucagon in fasted, fed, and hypoglycemic rats. Am J Physiol 264(6 Pt 1):E943–E950.
23. Young AA, Wang MW, Cooper GJS (1991): Amylin injection causes elevated plasma lactate and glucose in the rat. FEBS Lett 291:101–104.
24. Young AA, Wang MW, Cooper GJS, Mott DM (1991): Amylin and insulin exert complementary control over Cori cycle activity. J Cell Biochem Suppl 15 Part B:68.
25. Newgard CB, Hirsch LJ, Foster DW, McGarry JD (1983): Studies on the mechanism by which exogenous glucose is converted into liver glycogen in the rat. A direct or an indirect pathway? J Biol Chem 258:8046–8052.
26. Sugden MC, Watts DI, Palmer TN, Myles DD (1983): Direction of carbon flux in starvation and after refeeding: in vitro and in vivo effects of 3-mercaptopicolinate. Biochem Int 7:329–337.
27. Cooper GJS, Leighton B, Dimitriadis GD, Parry-Billings M, Kowalchuk JM, Howland K, Rothbard JB, Willis AC, Reid KB (1988): Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates glycogen metabolism in skeletal muscle. Proc Natl Acad Sci USA 85:7763–7766.
28. Lupien JR, Young AA (1993): No measurable effect of amylin on lipolysis in either white or brown isolated adipocytes from rats. Diabet Nutr Metab 6:13–18.
29. Gedulin B, Larson E, Provost S, Koda J (1993): The selective amylin antagonist, AC187, enhances the insulin response during intravenous glucose tolerance tests in anesthetized rats. Diabetes 42(Suppl 1):229A.
30. Dégano P, Silvestre RA, Salas M, Peiro E, Marco J (1993): Amylin inhibits glucose-induced insulin secretion in a dose-dependent manner—Study in the perfused rat pancreas. Regul Peptides 43:91–96.
31. Ohsawa H, Kanatsuka A, Yamaguchi T, Makino H, Yoshida S (1989): Islet amyloid polypeptide inhibits glucose-stimulated insulin secretion from isolated rat pancreatic islets. Biochem Biophys Res Commun 160: 961–967.
32. Murakami M, Suzuki S, Sato Y, Shintami S, Abe S, Suzuki K, Ishuzuka J, Toyota T (1990): Effects of amylin on insulin secretion from RINm5F cells. Diabetes 39(Suppl 1):266A.
33. Wagoner PK, Chen C, Worley JF, Dukes ID (1993): Amylin modulates β-cell glucose sensing via effects on stimulus-secretion coupling. Proc Natl Acad Sci USA 90:9145–9149.

34. Wang Z, Bennet WM, Ghatei MA, Byfield PGH, Smith DM, Bloom SR (1993): Influence of islet amyloid polypeptide and the 8–37 fragment of islet amyloid polypeptide on insulin release from perifused rat islets. Diabetes 42:330–335.
35. Young AA, Carlo P, Rink TJ, Wang MW (1992): $^{8-37}$hCGRP, an amylin receptor antagonist, enhances the insulin response and perturbs the glucose response to infused arginine in anesthetized rats. Mol Cell Endocrinol 84:R1–5.
36. Batchelor BR, Stern JS, Johnson PR, Mahler RJ (1975): Effects of streptozotocin on glucose metabolism, insulin response, and adiposity in ob/ob mice. Metabolism 24: 77–91.
37. Mahler RJ, Szabo O (1971): Amelioration of insulin resistance in obese mice. Am J Physiol 221:980–983.
38. Young AA, Crocker L, Clarke H, Wolf-Lopez D, Rink T (1993): Streptozotocin treatment of Fatty Zucker rats reverses insulin resistance in soleus muscle. Diabetes 41(Suppl 1).
39. Young AA, Gedulin B, Gaeta LSL, Prickett KS, Beaumont K, Larson E, Rink T (submitted): Evidence for a hormonal role of amylin from metabolic effects of a selective antagonist in anesthetized rats.