Journal of Cellular Biochemistry 55S:19–28 (1994)

Molecular Physiology of Amylin

Richard A. Pittner, Keith Albrandt, Kevin Beaumont, Laurie S.L. Gaeta, Joy E. Koda, Candace X. Moore, Judith Rittenhouse, and Timothy J. Rink

Amylin Pharmaceuticals, Inc., San Diego, California 92121

Abstract Amylin is a 37-amino acid peptide first isolated, purified, and characterized from the amyloid deposits in the pancreases of type 2 diabetics. It is synthesized and secreted primarily from pancreatic beta cells along with insulin. The ability of amylin to potently reduce insulin-stimulated incorporation of glucose into glycogen in skeletal muscle requires both an intact 2Cys–7Cys disulfide bond and a COOH-terminal amide. Amylin has structural and functional relationships to two other messenger proteins, calcitonin and CGRP. Amylin has relatively potent calcitonin-like activity on bone metabolism and weaker CGRP-like activity on the vasculature. CGRP is a slightly weaker agonist than amylin for metabolic responses. Although rat calcitonins are weak, teleost fish calcitonins are very potent agonists for amylin's metabolic effects. This group of peptides appears to act on a family of related G protein-coupled receptors; several variant calcitonin receptors have recently been cloned and expressed. These receptors appear to be coupled to adenylyl cyclase in many instances; recent evidence supports the view that amylin's effects on skeletal muscle occur, at least in large part, through activation of the cAMP pathway.

Key words: amylin, calcitonin, CGRP, cAMP

INTRODUCTION

This brief overview outlines some key aspects of amylin structure, synthesis, secretion, and physiology that have been characterized during the 6 years since amylin was first identified [1]. A potent effect of amylin at inhibiting insulin-stimulated incorporation of glucose into glycogen in rat soleus muscle, was reported in 1988 [2]; since that time, biological actions on several metabolically important processes and a variety of other systems have been reported [3]. Functional relationships to calcitonin and CGRP (calcitonin gene-related peptide) have also been uncovered; the current understanding of these relationships and the emerging picture of a family of receptors of these bioactive peptides are discussed. Detailed investigation of receptor binding, signal transduction, and cellular target enzymes reveals very potent effects of amylin in vitro, in the 10–100-pM range, comparable to the levels of amylin reported in rodent and human plasma. These findings, along with the developing concepts of amylin's roles in metabolic control and the use of amylin receptor antagonists to probe actions of endogenous amylin in vivo (summarized in Young et al., page 12, this issue) fit with the original concept of amylin as an endocrine partner to insulin.

STRUCTURE

Figure 1 shows the primary structure of human amylin as directly elucidated by amino acid sequencing of material isolated and purified from human pancreatic amyloid, together with its two post-translational modifications, the disulfide bond and the COOH-terminal amidation [1]. Amylin is about 50% identical to CGRP-I and CGRP-II and structurally related to the calcitonins. Determination of the primary sequence allowed molecular cloning of the amylin gene in humans and several mammalian species. There appears to be only one gene copy, in humans on chromosome 12. The open reading frame codes for a precursor molecule, preproamylin with typical hormone-processing dibasic sites, and an amidation signal, i.e., a glycine next to the COOH-terminal tyrosine [4].

There is considerable interspecies variation in the central region of the primary structure of amylin. However, the N-terminal and C-terminal portions of the molecule are strongly conserved. In human amylin the region between amino acids 20 and 29 appears to form beta-

Received October 19, 1993; accepted January 5, 1994.

Address reprint requests to Dr. Timothy J. Rink, Amylin Pharmaceuticals, Inc., 9373 Towne Centre Drive, San Diego, CA 92121.

PROTEIN SEQUENCES ALIGNMENT

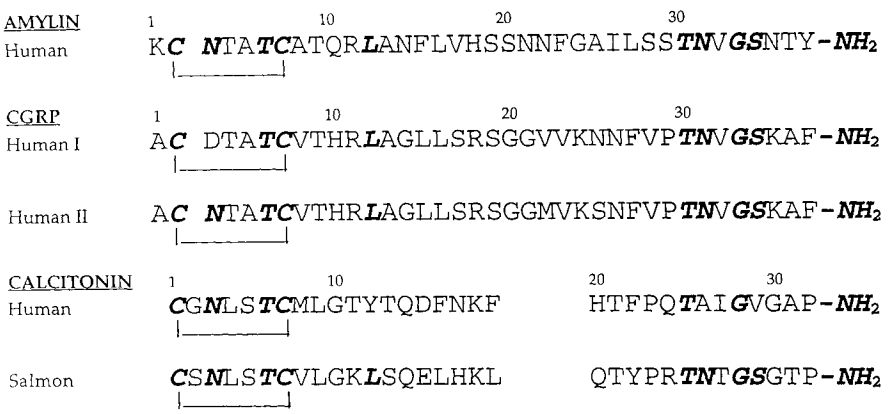

Fig. 1. Comparison of the structure of amylin, CGRPs, and calcitonins.

pleated sheets and to be responsible for the very strong tendency to self-aggregate and form the insoluble plaques, which are pancreatic amyloid material [5]. This feature makes human amylin extremely difficult to work with either as an experimental tool or as a potential therapeutic agent. For this reason, a modified amylin agonist, AC137, with proline substituted at positions 25, 28, and 29, is now being used in clinical trials.

Recent circular dichroism and solution NMR measurements (N. Anderson, manuscript in preparation) with both human and rat amylin indicate a significant amount of secondary structure in these molecules. The N-terminal loop has a fairly well-defined structure, the region from approximately amino acids 7–20 has a marked tendency to form an α-helix and from both of these physical chemistry studies and studies of chemical modifications of the COOH-terminus, there is good evidence for a β-turn between positions 30 and 36.

SECRETION

Tissue localization studies using in situ hybridization (mRNA) or immunofluorescence (ir) show that amylin message and protein are most prominent in pancreatic β-cells [6,7]; however, as shown in Table 1, evidence from one or both techniques indicates that small amounts of amylin may be expressed in a variety of other tissues [8]. Stimulus–secretion studies with isolated β-cells [9], disaggregated islets [10,11], and perfused pancreas [12] show that during brief stimulation by glucose or arginine, amylin, and insulin are secreted at approximately constant molar ratio, typically 2–5% amylin compared to insulin in the rat. However, on prolonged stimulation by glucose, and in models in which animals become insulin resistant and type 2 diabetic, there is evidence that the amylin/insulin ratio can increase [13,14]. Minimal or undetectable levels of amylin in human subjects with insulin-dependent diabetes, in whom β cells have been destroyed by autoimmune attack, suggest that most circulating amylin derives from β-cell secretion [15].

TABLE I. Amylin Localization

High	Pancreatic β-cell	ir, mRNA
	Pancreatic δ-cell (rat)	ir
Low	Dorsal root ganglion	ir, mRNA
	Lung	ir, mRNA
	Stomach	ir, mRNA
	Intestinal tract	ir
	Hypothalamus	ir
Tumors	Insulinoma	ir, mRNA
	Osteoblastoma	ir, mRNA
	Pancreatic tumors	ir
	Oat cell carcinoma	ir

In humans, amylin levels measured by radioimmunoassay range from 2 to 10 pM in the fasted state to 8–20 pM postprandially or post-glucose [15–17]. In insulin-resistant states, including obesity, glucose intolerance, and gestational diabetes, considerably higher levels are often seen, with fasting levels as high as 40 pM and postprandial levels as high as 90 pM, has been seen in a few subjects [18]. In rodents, reported amylin levels are somewhat higher,

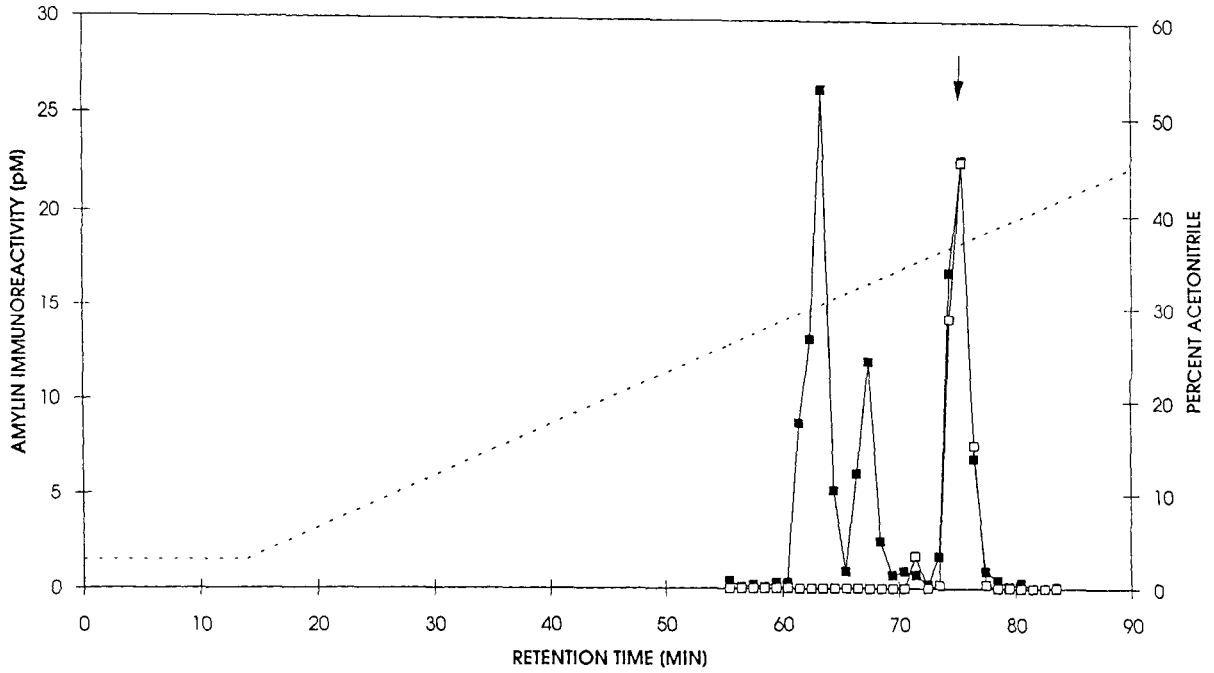

Fig. 2. Reversed-phase HPLC of plasma amylin immunoreactivity. 20 ml of plasma from a normal subject after overnight fasting was acidified by addition of an equal volume of 1% trifluoroacetic acid (TFA) and extracted on a SepPak C18 column with elution at 25–50% CH_3CN in 0.5% TFA. The partially evaporated eluate was fractionated on a Vydak C4 300A 4.6 × 250 mm reversed-phase column at 0.6 ml/min with an acetonitrile gradient in 0.1% TFA. Fractions collected at 1 min intervals were lyophilized, resuspended in 0.25-ml assay buffer, and assayed for amylin immunoreactivity using synthetic human amylin standards. Open squares:immunoreactivity for full length amylin; closed squares:immunoreactivity recognizing amylin mid-to-amidated COOH-terminal region.

reaching several hundred picomoles (pM) in a number of insulin-resistant varieties [19,20].

Significant proportions of circulating immunoreactive amylin have been reported to consist of fragments or altered species that do not coelute with intact synthetic amylin during reversed-phase chromatography [21,22]. Recently, we have developed a series of two-site monoclonal assays with one of the pair of monoclonals specific for the amidated COOH-terminal end of the molecule and the other directed toward either the middle region or the NH_2-terminal (Phelps et al. and Percy et al., manuscripts in preparation). The data for healthy human subjects (Fig. 2), indicate that approximately 40% of circulating amylin-like immunoreactivity is in the intact molecule, while some 60% is distributed among other molecular species, which, based on antibody-recognition patterns, contain alterations near the amino-terminus. These amylin-like immunoreactive species may be secreted by the pancreas, since peak interconversion was not observed upon incubation with plasma, and similar immunoreactive peaks have been found in perfusate from isolated human pancreas (Rittenhouse et al., manuscript in preparation). The task now is to isolate and chemically characterize these different fractions, a nontrivial undertaking in view of the very low plasma levels of these molecules.

IN VITRO AMYLIN ACTIONS ON METABOLICALLY RELEVANT TISSUES

Skeletal Muscle

The first demonstrated biologic action of amylin was the inhibition of insulin-stimulated incorporation of labeled glucose into glycogen in skeletal muscle [2,23]. Subsequent studies have confirmed and extended these findings; thus, it is now clear that this is a highly potent effect, half-maximal at sub-nanomolar concentrations in the rat soleus muscle [24] (see also Young et al., page 12, this issue) and with measurable effects at 10–100 pM [25]. The available published data indicate that amylin influences muscle glycogen metabolism via activation of glycogen phosphorylase and inhibition of glycogen synthase [26–29], enzymatic changes known to follow elevation of cytosolic calcium during

contraction and elevation of cAMP by, for instance, circulating epinephrine. The consequences of these enzyme modulations include reduction of total glycogen content and an increase lactate production, due to flux of glucose 6-P through the glycolytic pathway, in the absence of any stimulus to increase oxidative phosphorylation [23,28,29]. Whether amylin has other molecular targets in muscle, such as glucose transport [28,30–32] is not clear; but amylin appears not to interact with insulin receptors at the level of binding or of their tyrosine kinase activity [33,34].

Fat and Liver

Amylin appears to have no direct actions on adipocytes, at least in terms of influencing glucose uptake, lipogenesis, or lipolysis, whether under basal conditions or in the presence of insulin or norepinephrine [2,35]. It has proved difficult despite efforts by several different groups, including our own, to demonstrate convincing reproducible effects of amylin on isolated hepatocytes. Gomez-Foix et al. [36] reported that amylin inhibited the ability of insulin to modify glycogen metabolism, whilst having no direct effect itself. Insulin stimulated glucokinase gene expression however, is unaffected by amylin [37]. Glucagon-like glycogenolytic effects of amylin have been reported by some investigators [38,39], but not by others [40]. Interestingly, Stephens et al. [40] also demonstrated amylin/CGRP binding and effects on cAMP only in nonparenchymal cells. Studies using perfused livers have also failed to demonstrate any glucose producing effects of amylin [41,42]. Some investigators [43], have observed small rises in cAMP (as compared to the effects of glucagon) and modest activation of phosphorylase in purified hepatocytes challenged with high nanomolar levels of amylin, but only in the presence of IBMX to block cyclic nucleotide phosphodiesterases. The physiologic relevance of this action is debatable, since the reported actions of amylin on liver metabolism in vivo are more readily associated with increased glycogen synthesis [44], as noted in Young et al., page 12, this issue. However, the available data do not exclude some direct action of amylin on liver cells and further investigation in vitro and in vivo is needed.

Pancreatic β-Cells

The available evidence supports the idea that amylin can reduce insulin secretion, by an action at the level of the islet and very likely at the β-cell itself. Amylin, at concentrations as low as 75 pM, reduced markedly glucose-stimulated insulin secretion in the perfused rat pancreas [45,46], and similar effects are reported with perfused islets and disaggregated mouse β-cells [47–49] and Rin m5F cells [38]. This inhibition appears not to involve β-cell function [46] or to be mediated by somatostatin [50]. Others have failed to show an effect of amylin on insulin release [51–53]. This discrepancy could be due to endogenously released amylin from islets or cells in vitro that may be sufficient to mask the effects of exogenously added amylin. Another approach for these studies is to block the effects of endogenously secreted amylin, by the use of specific amylin antagonists. Indeed, $^{8-37}$hCGRP [54] and $^{8-37}$amylin [49] have been reported to increase insulin secretion in whole animals and in isolated islets, and we have recently found similar effects with a β-cell line (HIT cells) in static culture (Moore et al., unpublished observations). A newly identified amylin receptor antagonist (AC66) which is highly selective for amylin but not $CGRP_1$ receptors (Beaumont, Gaeta, Young, Wang et al., unpublished results) produced similar effects. These results are consistent with endogenously secreted amylin exerting an inhibitory effect on β-cell insulin secretion which is reversed by an amylin antagonist.

Bone and Vasculature Tissue

Amylin is also reported to have relatively potent effects (in the nM range), inducing quiescence in isolated osteoclasts [55], consistent with activation of calcitonin receptors linked to adenylyl cyclase [56,57]. Various other cellular effects of amylin are reported and have been recently reviewed [3,58] but are either not germane to metabolic regulation or are not readily reproducible, at least in our hands. While amylin evidently has weak CGRP-like vasodilator and hypotensive actions in vivo [59,60], we are not aware of published in vitro investigations of amylin action on the vasculature.

AMYLIN-LIKE ACTIONS OF CGRP AND CALCITONINS

The ability of amylin to mimic, in part at least, the best recognized actions of CGRP and calcitonins has been alluded to above. Further indicating a functional relationship, these peptides have been found to mimic amylin's metabolic actions. It was shown during the earliest studies of amylin, that CGRP was almost as potent as amylin in influencing glycogen metabolism in skeletal

muscle [23], and CGRP was reported to inhibit insulin secretion [61–63]. Despite the apparent abundance of CGRP receptors in liver membranes [64–66], CGRP appears, like amylin, to have only very modest effects on liver metabolism [36,40,43]. We are not aware of studies of CGRP on adipocytes. In vivo, CGRP mimics amylin's actions to elevate plasma lactate and glucose [60,67,68]. When one attempts to allow for the marked cardiovascular action of CGRP it appears that higher doses of exogenous CGRP than of amylin are required to evoke measurable increases in plasma lactate and glucose in fasted anesthetized rats; thus, CGRP appears to be less potent than amylin on these metabolic effects [60].

A surprising finding was that salmon calcitonin inhibited insulin-stimulated incorporation of glucose into glycogen in isolated rat soleus muscle more potently than does rat amylin; by contrast rat calcitonin is distinctly less potent [3]. In vivo studies fit with the in vitro data in that salmon calcitonin increases plasma lactate and glucose concentrations in fasted anesthetized rats more potently than does amylin [3] (Young et al., page 12, this issue). As with CGRP, it was reported some years ago that salmon calcitonin can inhibit insulin secretion [69,70]. Thus salmon calcitonin shares the most evident metabolic actions of amylin. Table 2 summarizes some of our current thinking about the functional relations of this family of peptides.

RECEPTORS

The in vitro and in vivo functional studies of amylin, calcitonin and CGRP action most likely reflect interaction of these peptides, with differing selectivities at a family of receptors. We have used the phrase "receptor cross-talk" to encompass this notion.

CGRP

There is a quite extensive literature on CGRP-binding studies, with rather less work on functional pharmacological dose–response relations [71]. Space precludes reviewing these even briefly, but we believe most readers would conclude that there are at least two sub-types of CGRP receptors; for example those in vascular smooth muscle, liver and cerebella membranes appear pharmacologically distinct from CGRP receptors on endothelial cells, atrium, or vas deferens, in terms of relative apparent affinity for CGRP-I and -II and for (8–37)-CGRP. There is a quite high density of CGRP receptors in skeletal muscle membranes which may account for the marked CGRP-responsiveness of this tissue [66]. Indeed, CGRP receptors are wide spread, and are found on many cell lines [72,73], consistent with the wide distribution of CGRP as a neurotransmitter (or neuromodulator) in both the central and peripheral nervous systems. Many [65,66] but not all [74] CGRP receptors appear to couple to adenylyl cyclase via Gs. Amylin typically displaces, or competes for, radiolabeled CGRP at these receptors with an affinity 100–1,000 times lower than does cold CGRP [65,72,73]. Calcitonins, whether mammalian or teleost have shown weak interaction with CGRP receptors.

TABLE II. Biologic Actions of Amylin, Calcitonin and CGRP

	Skeletal muscle	β-cells	Vascular	Bone
Amylin	+++	+++	+	++(+)
Rat calcitonin	+	?	–	+++
Salmon calcitonin	++++	+++	–	++++
CGRP	++	+++	++++	++

Calcitonin

It has seemed clear from binding studies that there are at least two rather different calcitonin receptors [55,75–77]. Several cell lines, including some from human breast cancers, display dense calcitonin binding with salmon calcitonin, some 10–50-fold more potent than mammalian calcitonin [72,78]. It seems generally accepted, that these receptors are representative of those on osteoclasts, that subserve calcitonin's major actions on bone and calcium metabolism. Apparently different receptors are found, for instance, in certain brain regions [79] and in renal cortex [80], with pM affinity for salmon calcitonin and only μM affinity for mammalian calcitonin. The finding of such receptors together with reports of various tissue extracts with salmon calcitonin-like immunoreactivity [81–84] has suggested to some authors that there may be as-yet unidentified endogenous ligands in mammals directed to this type of calcitonin receptor. Perhaps reflective of the multiplicity of calcitonin receptors, amylin, and to a lesser extent CGRP, appears to fairly potently interact with certain high affinity calcitonin binding sites [55–57,86], while both amylin and CGRP interact much more weakly than calcitonin at others [85]. Consistent with this multiplicity of pharmacological profiles for calcitonin binding, recent molecular cloning

studies are revealing multiple structural variants of calcitonin receptors [87–89].

The distinct spectrum of biologic actions of amylin and our analysis of dose–response relations were considered by our group to support the concept of distinct amylin receptors, although several other groups have concluded that amylin's metabolic effects are mediated via CGRP receptors [43,65,66,90]. In part, their conclusion reflected their failure to identify high-affinity amylin binding sites. We have now reported the presence of high-affinity, Kd 27 pM amylin binding in certain areas of rat brain as assessed by both autoradiography and binding of 125I-Bolton-Hunter-labeled rat amylin to membrane preparations [91]. The highest concentration of these binding sites, which we believe to be receptors, is in the nucleus accumbens. Amylin binding sites are also present in certain other regions including the OVLT and other brain regions outside the blood-brain barrier and thus, potentially well placed to respond to fluctuations of amylin concentration in the circulation.

Thus far, there are no reports of molecular cloning of a high-affinity receptor with pharmacological characteristics that fit with the binding and competition profile of the rat nucleus accumbens receptor and the pharmacological profile of amylin agonists and antagonists observed in the soleus muscle preparation. Thus, the amylin receptor has been characterized pharmacologically, as almost all the well recognized receptors have been, prior to their molecular cloning. Practically speaking, one can make considerable progress with this approach including identifying numerous potent and selective amylin agonists and antagonists.

SIGNAL TRANSDUCTION

Recent reports [27,33] stated that amylin does not raise cAMP in skeletal muscle. However amylin is reported to activate cyclase in membranes containing CGRP receptors [33,43,65,90], albeit at nM concentrations, probably reflecting action at CGRP receptors. Amylin actions on osteoclasts, perhaps via certain calcitonin receptors appear to be mediated by cAMP [56,57]. Thus one might expect the effects of amylin to be mediated via activation of adenylyl cyclase in other situations including skeletal muscle, more particularly since amylin's actions on glycogen metabolism are consistent with transduction via cAMP signaling pathways.

We believe that there is now persuasive evidence from our own and other laboratories showing that amylin does potently increase cAMP in rat soleus muscle [25,92] (Pittner et al., manuscript in preparation) in a dose-dependent manner, as shown in Figure 3. This dose-response is compatible with cAMP as the link to the observed changes in glycogen metabolism [24]. It is also reported that amylin in the 10–100-pM range stimulates activation of cAMP-dependent protein kinase in soleus muscle [25], and we find dose-dependent activation of adenylyl cyclase by amylin in membrane fractions from rat [93], and human skeletal muscle (Moore et al., unpublished observations). The molecular mechanism by which amylin reduces insulin secretion by β-cells is unknown. It has been suggested that amylin causes membrane hyperpolarization in the isolated β-cell [46] and decreases cAMP levels in Rin m5F cells through pertussis toxin-sensitive Gi proteins [38].

CELLULAR TARGETS

The cellular consequences of amylin's action have been explored in some detail only in skeletal muscle, where it is evident that amylin activates glycogen phosphorylase leading to lactate production (via glycolysis) and appears to inhibit glycogen synthase activity [23,26–29]. The dose-dependent activation of phosphorylase in soleus muscle and loss of glycogen mass evoked by amylin parallels the inhibition of insulin stimu-

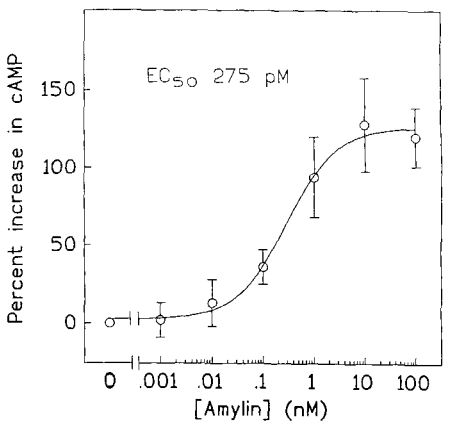

Fig. 3. Effects of rat amylin on cyclic AMP levels in soleus muscle. Soleus muscles were prepared and incubated essentially as described by Young [24]. Following a 30-min preincubation, rat amylin was added at the concentrations indicated in the absence of IBMX and muscle pieces were further incubated for 10 min. The cyclic AMP content of the muscle was subsequently determined by RIA. Results are means ± SEM from 6 muscle pieces.

lated incorporation of radiolabelled glucose into glycogen (Pittner et al., manuscript in preparation).

It is not known whether amylin has other cellular targets in skeletal muscle. The reported data on glucose transport are conflicting [28,30–32]. One can question whether physiologically meaningful glucose transport studies can be obtained in isolated muscle pieces, which are very dependent on muscle size, shape, and diffusion distance [95], or L6 myoblasts, which express very low levels of GLUT4, the insulin-sensitive glucose transporter found in skeletal muscle [96]. Epinephrine, which also activates a cAMP signaling system, has small effects on skeletal muscle glucose fluxes, compared to the effects of insulin [94,96]. Inhibition of 2-deoxyglucose, but not 3-O-methylglucose transport as reported by Young et al. [28], would suggest that inhibition of glucose uptake is due to indirect inhibition of hexokinase activity because of elevated glucose 6-phosphate concentrations following amylin (or for that matter CGRP or epinephrine) stimulation of glycogenolysis, rather than specific inhibition of a glucose transporter.

SUMMARY

Since its discovery 6 years ago, a large body of evidence has accumulated that persuasively supports the concept of amylin as an endocrine hormone involved in metabolic regulation and that excess amylin action contributes to the progression of insulin resistance. As the biological actions of amylin in other metabolic areas become apparent, a functional relationship to calcitonin and CGRP has been uncovered, and it seems likely that these peptides exert their biological actions via a family of related G protein-coupled receptors.

REFERENCES

1. Cooper GJS, Willis AC, Clark A, Turner RC, Sim RB, Reid KBM (1987): Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. Proc Natl Acad Sci USA 84:8628–8632.
2. Cooper GJS, Leighton B, Dimitriadis GD, Parry-Billings M, Kowalchuk JM, Howland K, Rothbard JB, Willis AC, Reid KBM (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.
3. Rink TJ, Beaumont K, Koda J, Young AA (1993): Structure and biology of amylin. Trends Pharmacol Sci 14: 113–118.
4. Cooper GJS, Day AJ, Willis AC, Roberts AN, Reid KBM, Leighton B (1989): Amylin and the amylin gene: Structure, function and relationship to islet amyloid and to diabetes mellitus. Biochim Biophys Acta 1014:247–258.
5. Glenner GG, Eanes D, Wiley CA (1988): Amyloid fibrils formed from a fragment of the pancreatic islet amyloid protein. Biochem Biophys Res Commun 155:608–614.
6. Johnson KH, O'Brien TD, Hayden DW, Jordan K, Ghobrial HKG, Mahoney WC, Westermark P (1988): Immunolocalization of islet amyloid polypeptide (IAPP) in pancreatic beta cells by means of peroxidase–antiperoxidase (PAP) and protein A–gold techniques. Am J Pathol 130:1–8.
7. Leffert JD, Newgard CB, Okamoto H, Milburn JL, Luskey KL (1989): Rat amylin: Cloning and tissue specific expression in pancreatic islets. Proc Natl Acad Sci USA 86:3127–3130.
8. Ferrier GJM, Pierson AM, Jones PM, Bloom SR, Girgis SI, Legon S (1989): Expression of the rat amylin (IAPP/DAP) gene. J Endocrinol 3:R1–R4.
9. Moore CX, Cooper GJS (1991): Co-secretion of amylin and insulin from cultured islet beta cell. Biochem Biophys Res Commun 179:1–9.
10. Kanatsuka A, Makino H, Ohsawa H, Tokuyama Y, Yamaguchi T, Yoshida S, Adachi M (1989): Secretion of islet amyloid polypeptide in response to glucose. FEBS Lett 259:199–201.
11. Kahn SE, D'Alessio DA, Schwartz MW, Fujimoto WY, Ensinck JW, Taborsky GJ, Porte D (1990): Evidence of co-secretion of islet amyloid polypeptide and insulin by beta cells. Diabetes 39:639–642.
12. Ogawa A, Harris V, McCorkie SK, Unger RH, Luskey KL (1990): Amylin secretion from rat pancreas and its selective loss after streptozotocin treatment. J Clin Invest 85:425–429.
13. O'Brien TD, Westermark P, Johnson KH (1991): Islet amyloid polypeptide and insulin secretion from isolated perfused pancreas of fed, fasted, glucose-treated, and dexamethasone-treated rats. Diabetes 40:1701–1706.
14. Gedulin B, Cooper GJS, Young AA (1991): Amylin secretion from the perfused pancreas: Dissociation from insulin and abnormal elevation in insulin resistant diabetic rats. Biochem Biophys Res Commun 180:782–789.
15. Koda JE, Fineman M, Rink TJ, Dailey GE, Muchmore DB, Linarelli LG (1992): Amylin concentrations and glucose control. Lancet 339:1179–1180.
16. Hartter E, Svoboda T, Ludvik B, Schuller M, Lell B, Kuenberg E, Brunnbaeur M, Woloszczuk W, Prager R (1991): Basal and stimulated plasma levels of pancreatic amylin indicate its co-secretion with insulin in humans. Diabetologia 34:52–54.
17. Sanke T, Hanabusa T, Nakano Y, Oki C, Okai K, Nishimura S, Konodo M, Nanjo K (1991): Plasma islet amyloid polypeptide (amylin) levels and their responses to oral glucose in type 2 (non-insulin-dependent) diabetic patients. Diabetologia 34:129–132.
18. Zweers EJK, Bravenboer B, van Hulst KL, Lips CJM, Christiaens GCML, Hackeng WHL, Erkelens DW (1992): Glucose stimulated islet amyloid polypeptide and gestational diabetes mellitus. Diabetologia 35:A179.
19. Huang HJS, Young AA, Koda JE, Tulp OL, Johnson MJ, Cooper GJS (1991): Hyperamylinemia, hyperinsulinemia and insulin resistance in genetically obese LA/N-cp rats. Hypertension 19:101–109.

20. 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.
21. Nakazato M, Miyazato M, Asai J, Mitsukawa T, Kangawa K, Matsuo H, Matsukura S (1990): Islet amyloid polypeptide, a novel pancreatic peptide, is a circulating hormone secreted under glucose stimulation. Biochem Biophys Res Commun 169:713–718.
22. Shiomi K, Nakazato M, Miyazato M, Kangawa K, Matsuo H, Matsukura S (1992): Establishment of hypersensitive radioimmunoassay for islet amyloid polypeptide using antiserum specific for its N-terminal region. Biochem Biophys Res Commun 186:1065–1073.
23. Leighton B, Copper GJS (1988): Pancreatic amylin and calcitonin gene related peptide cause resistance to insulin in skeletal muscle in vitro. Nature 335:632–635.
24. Young AA, Gedulin B, Wolfe-Lopez D, Greene HE, Rink TJ, Cooper GJS (1992): Amylin and insulin in rat soleus muscle: Dose responses for co-secreted non-competitive antagonists. Am J Physiol 263:E274–E281.
25. Weiel J, Ryde EH, Irsula O, Frangakis C (1993): Activation of PKa by amylin in skeletal muscle. Diabetes 42:A410.
26. 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.
27. Young AA, Mott DM, Stone K, Cooper GJS (1991) Amylin activates glycogen phosphorylase in the isolated soleus muscle of the rat. FEBS Lett 282:149–151.
28. 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: E457–E461.
29. Frontoni S, Choi SB, Banduch D, Rossetti L (1991): In vivo insulin resistance induced by amylin primarily through inhibition of insulin-stimulated glycogen synthesis in skeletal muscle. Diabetes 40:568–573.
30. 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.
31. Kreutter DK, Orena SJ, Torchia AJ, Contillo LG, Andrews GC, Stevenson RW (1993): Amylin and CGRP induce insulin resistance via a receptor distinct from cAMP-coupled CGRP receptor. Am J Physiol 264:E606–E613.
32. 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 vivo incubated muscle strips. Diabetologia 35:26–31.
33. Koopmans SJ, Van Mansfield ADM, Jansz HMJ, Radder JK, Frolich M, De Boer SF, Kreutter DM, Andrews GC, Maassen JA (1991): Amylin-induced resistance in conscious rats: The liver is more sensitive to amylin than peripheral tissues. Diabetologia 34:218–224.
34. Follett L, Bruer-Ash M (1991) Effect of amylin on insulin receptor kinase activity in vivo in the rat. Clin Res 39:39A.
35. Lupien JR, Young AA (1992): No measurable effect of amylin on lipolysis in either white or brown isolated adipocytes from rats. Diabetes Nutr Metab 6:1–6.
36. Gomez-Foix AM, Rodriguez-Gil JE, Guinovart JJ (1991): Anti-insulin effects of amylin and CGRP on hepatic glycogen metabolism. Biochem J 276:607–610.
37. Nouspikel T, Gjinovci A, Li S, Iynedjian PB (1992): Unimpaired effect of insulin on glucokinase gene expression in hepatocytes challenged with amylin. FEBS Lett 301:115–118.
38. Suzuki S, Muramaki M, Abe S, Satoh Y, Shintani S, Ishizuka J, Suzuki K, Thompson JC, Toyota T (1992): The effects of amylin on insulin secretion from Rin m5F cells and glycogen synthesis and lipogenesis in rat primary cultured hepatocytes. Diabetes Res Clin Pract 15:77–84.
39. Ciaraldi TP, Goldberg M, Odom R, Stolpe M (1992): In vitro effects of amylin on carbohydrate metabolism in liver cells. Diabetes 41:975–981.
40. Stephens TW, Heath WF, Hermeling RN (1991): Presence of liver CGRP/amylin receptors in only nonparenchymal cells and absence of direct regulation of rat liver glucose metabolism by CGRP/amylin. Diabetes 40:395–400.
41. Nishimura S, Sanke T, Machida K, Bessho H, Hanabusa T, Nakai K, Nanjo K (1992): Lack of effect of islet amyloid polypeptide on hepatic glucose output in the in situ perfused rat liver. Metabolism 41:431–434.
42. Roden M, Liener K, Furnsinn C, Nowotny P, Hollenstein U, Vierhapper H, Waldhausl W (1992): Effects of amyloid polypeptide on hepatic insulin resistance and glucose production in the isolated perfused rat liver. Diabetologia 35:116–120.
43. Bushfield M, Savage A, Morris NJ, Houslay MD (1993): A mneminical or negative co-operativity model for the activation of adenylate cyclase by a common G-protein coupled calcitonin gene-related peptide (CGRP)/amylin receptor. Biochem J 293:229–236.
44. Young AA, Crocker LB, Wolfe-Lopez D, Cooper GJS (1991): Daily amylin replacement reverses hepatic glycogen depletion in insulin-treated streptozotocin diabetic rats. FEBS Lett 287:203–205.
45. Degano 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 pancreas. Regul Pept 43:91–96.
46. Silvestre RA, Peiro E, Degano P, Miralles P, Marco J (1990): Inhibitory effect of rat amylin on the insulin responses to glucose and arginine in the perfused rat pancreas. Regul Pept 31:23–31.
47. Wagoner PK, Chen C, Worley JF, Dukes ID, Oxford GS (1993): Amylin modulates β-cell glucose sensing via effects on stimulus–secretion coupling. Proc Natl Acad Sci USA 90:9145–9149.
48. Ohsawa H, Kanatsuka A, Tokuyama Y, Yamaguchi T, Makino H, Yoshida S (1989): Islet amyloid polypeptide inhibits glucose-stimulated insulin secretion from isolated rat pancreatic islets. Biochem Biophys Res Commun 124:45–53.
49. 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.
50. Peiro E, Degano P, Silvestre RA, Marco J (1991): Inhibition of insulin release by amylin is not mediated by changes in somatostatin output. Life Sci 49:761–765.

51. Bretherton-Watt D, Gilbey SG, Ghatei MA, Beacham J, Macrae AD, Bloom SR (1990): Failure to establish islet amyloid polypeptide (amylin) as a circulating beta cell inhibiting hormone. Diabetologia 33:115–117.
52. Broderick CL, Brooke GS, Dimarchi RD, Gold G (1991): Human and rat amylin have no effects on insulin secretion in isolated rat pancreatic islets. Biochem Biophys Res Commun 177:932–938.
53. Pettersson M, Ahren B (1990): Failure of islet amyloid polypeptide to inhibit basal and glucose stimulated insulin secretion in model experiments in mice and rats. Acta Physiol Scand 138:389–394.
54. Young AA, Carlo P, Rink TJ, Wang MW (1992): Infusion of 8-37hCGRP, an amylin antagonist, enhances the plasma insulin response and perturbs the plasma glucose response to an arginine challenge in anesthetized rats. Mol Cell Endocrinol 84:R1–R5.
55. Zaidi M, Moonga BS, Bevis PJR, Bascal ZA, Breimer LH (1990): The calcitonin gene peptides: Biology and clinical relevance. Crit Rev Clin Lab Sci 28:109–174.
56. Alam ASMT, Moonga BS, Bevis PJR, Huang CLH, Zaidi M (1991): Selective antagonism of calcitonin-induced osteoclastic quiescence (Q effect) by human calcitonin-gene related peptide (8–37). Biochem Biophys Res Commun 175:134–139.
57. Alam ASMT, Moonga BS, Bevis PJR, Huang CLH, Zaidi M (1993): Amylin inhibits bone resorption by a direct effect on the motility of rat osteoclasts. Exp Physiol 78:183–196.
58. Johnson KH, O'Brien TD, Betsholtz C, Westermark P (1992): Islet amyloid polypeptide: mechanisms of amyloidogenesis in the pancreatic islets and potential roles in diabetes mellitus. Lab Invest 66:522–535.
59. Brain SD, MacIntyre, I, Wimalwansa S, Williams TJ (1990): Amylin amide, which is structurally related to calcitonin gene-related peptides (CGRP), stimulates increased blood flow in vivo. Eur J Pharmacol 183:2221.
60. Young AA, Rink TJ, Wang MW (1993): Dose dependent characteristics for the hyperglycemic, hyperlactemic, hypotensive and hypocalcemic actions of amylin and calcitonin gene-related peptide-1 (CGRP-alpha) in the fasted, anaesthetized rat. Life Sci 52:1717–1726.
61. Ahren B, Martenssn H, Nobin A (1987): Effects of calcitonin gene-related peptide (CGRP) on islet hormone secretion in the pig. Diabetologia 6:1–15.
62. Koigre M, Ishizuka J, Thompson JC, Greeley GH (1991): Inhibitory action of islet amyloid polypeptide and calcitonin gene-related peptide on release of insulin from the isolated perfused rat pancreas. Pancreas 6:459–463.
63. Silvestre RA, Salas M, Degano P, Peiro E, Marco J (1993): Reversal of the inhibitory effects of calcitonin gene-related peptide (CGRP) and amylin on insulin secretion by the 8–37 fragment of human CGRP. Biochem Pharmacol 45:2343–2347.
64. Yamaguchi A, Chiba T, Yamatani T, Inui T, Morishita T, Nakamura A, Kadowaki S, Fukase M, Fujita T (1988): Calcitonin gene-related peptide stimulates adenylate cyclase activation via a guanine nucleotide-dependent process in rat liver plasma membranes. Endocrinology 123: 2591–2596.
65. Morishita T, Yamaguchi A, Fujita T, Chiba T (1990): Activation of adenylate cyclase by islet amyloid polypeptide with COOH-terminal amide via calcitonin gene-related peptide receptors on rat liver plasma membranes. Diabetes 39:875–877.
66. Chantry A, Leighton B, Day AJ (1991): Cross-reactivity of amylin with calcitonin gene-related peptide binding sites in rat liver and skeletal muscle membranes. Biochem J 277:139–143.
67. Yamaguchi A, Chiba T, Morishita T, Nakamura A, Inui T, Yamatani T, Kadowacki S, Chihara K, Fukase M, Fujita T (1990): Calcitonin gene-related peptide and induction of hyperglycemia in conscious rats in vivo. Diabetes 39:168–174.
68. Choi SB, Frontoni S, Rossetti L (1991): Mechanism by which calcitonin gene related peptide antagonizes insulin action in vivo. Am J Physiol 260:E321–E325.
69. Petralito A, Lunetta M, Liuzzo A, Fiore CE, Heynen G (1979): Effects of salmon calcitonin on blood glucose and insulin levels under basal conditions after intravenous glucose load. J Endocrinol Invest 2:209–211.
70. Lunetta M, Infantone E, Spanti D, Mughini L (1981): Effects of synthetic salmon calcitonin administration on gastrin, immunoreactive insulin and growth hormone release after protein meal in uremic patients. J Endocrinol Invest 4:185–188.
71. Tache Y, Holzer P, Rosenfield MG (eds) (1992): Calcitonin gene-related peptide: The first decade of a novel pleiotropic neuropeptide. Ann NY Acad Sci Vol. 657.
72. Muff R, Stangl D, Born W, Fischer JA (1992): Comparison of a calcitonin gene-related peptide receptor in a human neuroblastoma cell line (SK-N-MC) and a calcitonin receptor in a human breast carcinoma cell line (T47D). Ann NY Acad Sci 657:106–116.
73. Poyner DR, Andrew DP, Brown D, Bose C, Hanley MR (1992): Characterization of a receptor for calcitonin gene-related peptide on rat, L6 myocytes. Br J Pharmacol 105:441–447.
74. Goltzmann D, Mitchell J (1985): Interaction of calcitonin and calcitonin gene-related peptide at receptor sites in target tissues. Science 227:1343–1345.
75. Nakamuta H, Orlowski RC, Epand RM (1990): Evidence for calcitonin heterogeneity: binding studies with nonhelical analogs. Endocrinology 127:163–169.
76. Yates AJ, Guiterrez GE, Garrett IR, Mencel JJ, Nuss GW, Schreiber AB, Mundy GR (1990): A noncyclical analog of salmon calcitonin (N alpha-propionyl Di-Ala1,7,des-Leu19 sCT) retains full potency without inducing anorexia in rats. Endocrinology 126:2845–2849.
77. Twery MJ, Joels M, Gallagher IR, Orlowski RC, Moss RL (1988): Neuronal membrane sensitivity to a salmon calcitonin analogue with negligible ability to lower serum calcium. Neurosci Lett 86:82–88.
78. Findlay DM, Michelangeli VP, Eisman JA, Frampton RJ, Moselely JM, MacIntyre I, Whitehead R, Martin TJ (1980): Calcitonin and 1,25-dihydroxyvitamin D3 receptors in human breast cancer cell lines. Cancer Res 40:4764–4767.
79. Henke H, Tobler PA, Fischer JA (1983): Localization of salmon calcitonin binding sites in rat brain by autoradiography. Brain Res 272:373–377.
80. Sexton PM, Adam WR, Moselely JM, Martin TJ, Mendelsohn FAO (1987): Localization and characterization of renal calcitonin receptor by in vitro autoradiography. Kidney Int 32:862–868.
81. Fischer JA, Tobler PH, Henke H, Tschopp FA (1983): Salmon and human calcitonin-like peptides coexist in the human thyroid and brain. J Clin Endocrin Metab 57:1314–1316.

82. Shah GV, Noble MJ, Austenfield M, Weigel J, Deftos LJ, Mebust WK (1992): Presence of calcitonin-like immunoreactivity (iCT) in human prostate gland: Evidence for iCT secretion by cultured prostate cells. Prostate 21:87–97.
83. Shah GV, Deftos LJ, Crowley WR (1993): Synthesis and release of calcitonin-like immunoreactivity by anterior pituitary cells: Evidence for a role in paracrine regulation of prolactin secretion. Endocrinology 132:1367–1372.
84. Sexton PM, Hill JM (1992): Biologically active salmon calcitonin-like peptide is present in rat brain. Brain Res 596:279–284.
85. Alam ASMT, Bax CMR, Shankar VS, Bax BE, Bevis PJR, Huang CLH, Moonga BS, Pazianas M, Zaidi M (1993): Further studies on the mode of action of calcitonin on isolated rat osteoclasts: Pharmacological evidence for a second site mediating intracellular Ca+ mobilization and cell retraction. J Endocrinol 136:7–15.
86. D'Santos CS, Gatti A, Poyner DR, Hanley MR (1992): Stimulation of adenylate cyclase by amylin in CHO-K1 cells. Mol Pharmacol 41:894–899.
87. Albrandt K, Mull E, Brady MG, Herich J, Moore CX, Beaumont K (1993): Molecular cloning of two receptors from rat brain with high affinity for salmon calcitonin. FEBS Lett 325:225–232.
88. Lin HY, Harris TL, Flannery MS, Aruffo A, Kaji EH, Gorn A, Kowakowski LF, Lodish HF, Goldring SR (1991): Expression cloning of an adenylate cyclase-coupled calcitonin receptor. Science 254:1022–1024.
89. Gorn AH, Lin HY, Yamin M, Auron PE, Flannery MR, Tapp DR, Manning CA, Lodish HF, Krane SM, Goldring SR (1992): Cloning, characterization and expression of a human calcitonin receptor from an ovarian carcinoma cell line. J Clin Invest 90:1726–1735.
90. Zhu G, Dudley DT, Saltiel AR (1991): Amylin increases cyclic AMP formation in L6 myocytes through calcitonin gene-related peptide receptors. Biochem Biophys Res Commun 177:771–776.
91. Beaumont K, Kenney MA, Young AA, Rink TJ (1993): High affinity amylin binding sites in rat brain. Mol Pharmacol 44:493–497.
92. Stace PB, Fatania HR, Jackson A, Kerbey AL, Randle PJ (1992): Cyclic AMP and free fatty acids in the longer term regulation of pyruvate dehydrogenase kinase in rat soleus muscle. Biochim Biophys Acta 1135:201–206.
93. Moore CX, Rink TJ (1993): Amylin activates adenylate cyclase in rat soleus muscle. Diabetes 42:A821.
94. Clausen T, Flatman JA (1987): Effects of insulin and epinephrine on Na^+-K^+ and glucose transport in soleus muscle. Am J Physiol 252:E492–E499.
95. Henrikssen EJ, Holloszy JO (1991): Effect of diffusion distance on measurement of rat skeletal muscle glucose transport in vitro. Acta Physiol Scand 143:381–386.
96. Lawrence JC, Piper RC, Robinson LJ, James DE (1992): GLUT4 facilitates insulin stimulation and cAMP-mediated inhibition of glucose transport. Proc Natl Acad Sci USA 89:3493–3497.