Evidence that amylin stimulates lipolysis in vivo: a possible mediator of induced insulin resistance

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Ye, Ji-Ming, Megan Lim-Fraser, Gregory J. Cooney, Garth J. S. Cooper, Miguel A. Iglesias, David G. Watson, Bernard Choong, and Edward W. Kraegen. Evidence that amylin stimulates lipolysis in vivo: a possible mediator of induced insulin resistance. Am J Physiol Endocrinol Metab 280: E562–E569, 2001.—The present study investigated the role of amylin in lipid metabolism and its possible implications for insulin resistance. In 5- to 7-h-fasted conscious rats, infusion of rat amylin (5 nmol/h for 4 h) elevated plasma glucose, lactate, and insulin (P < 0.05 vs. control, repeated-measures ANOVA) with peak values occurring within 60 min. Despite the insulin rise, plasma nonesterified fatty acids (NEFA) and glycerol were also elevated (P < 0.001 vs. control), and these elevations (80% above basal) were sustained over the 4-h infusion period. Although unaltered in plasma, triglyceride content in liver was increased by 28% (P < 0.001) with a similar tendency in muscle (18%, P = 0.1). Infusion of the rat amylin antagonist amylin-(8–37) (125 nmol/h) induced opposite basal plasma changes to amylin, i.e., lowered plasma NEFA, glycerol, glucose, and insulin levels (all P < 0.05 vs. control); additionally, amylin-(8–37) blocked amylin-induced elevations of these parameters (P < 0.01). Treatment with acipimox (10 mg/kg), an anti-lipolytic agent, before or after amylin infusion blocked amylin’s effects on plasma NEFA, glycerol, and insulin but not on glucose and lactate. We conclude that amylin could exert a lipolytic-like action in vivo that is blocked by and is opposite to effects of its antagonist amylin-(8–37). Further studies are warranted to examine the physiological implications of lipid mobilization for amylin-induced insulin resistance.

amylin antagonist; fatty acids; glucose; acipimox

AMYLIN IS A 37-AMINO ACID peptide hormone co-released with insulin from pancreatic islet β-cells. It shares ~50% sequence identity with calcitonin gene-related peptide and has many similar biological actions (8, 17, 28). Amylin exerts a wide range of metabolic actions opposite to those of insulin, with the effects on glucose metabolism the most widely studied. In skeletal muscle, a major tissue for insulin-mediated glucose disposal, amylin potently opposes insulin-mediated glucose uptake by inhibiting glycogen synthesis and stimulating glycogenolysis (5, 20, 21, 25, 28, 36). The elevated circulating lactate from muscle glycogenolysis serves as a substrate in liver for gluconeogenesis, consistent with enhanced hepatic glucose production (28, 36).

In the rat, elevated plasma amylin levels are associated with various insulin resistance states (8, 17), such as in obese Zucker rats (8) and in normal rats treated with dexamethasone (10) or with human growth hormone (hGH) administration (15). Hyperamylinemia also accompanies human obesity (27) and type 2 (non-insulin-dependent) diabetes (30). Although there is controversy as to the exact physiological significance, some animal experiments point to a causal association between hyperamylinemia and insulin resistance. For example, amylin administration produces acute insulin resistance in rats characterized by hyperglycemia and hyperinsulinemia (2, 36). Amylin has been shown, by use of a hyperinsulinemic-euglycemic clamp technique, to impair insulin action in the rat (25). Recently, a type 2 diabetes-like state has been demonstrated in transgenic mice overexpressing human amylin (33).

Thus far, any involvement of amylin in insulin resistance has been attributed to its effects on glucose metabolism (5, 21, 25, 28). However, recent experiments in our laboratory demonstrated that amylin-(8–37), a truncated form of rat amylin, lowered plasma nonesterified fatty acid (NEFA) and glycerol in normal rats (25). In association with its lipid-lowering effects, amylin-(8–37) ameliorated acute hGH-induced insulin resistance where hyperamylinemia occurred (10). This might suggest an action of amylin on lipid metabolism that conceivably influences insulin sensitivity. However, although there is evidence that amylin-(8–37) acts generally as an antagonist of amylin (2, 34), it is difficult to be certain that specific actions are, in fact, related to and opposite to those of amylin. Adding to this uncertainty, literature reports of effects of amylin on lipid metabolism are scant. In two early studies performed in isolated rat adipocytes (9, 23), no effect of amylin was demonstrated on lipolysis or lipogenesis. This has led to a perception that amylin may not play

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a direct role in lipid metabolism (35). Although Suzuki et al. (32) showed a lipogenic effect of amylin in cultured rat hepatocytes, it is difficult to assess the implications of these results in vivo. We are unaware of any studies examining effects of amylin itself on in vivo lipid metabolism. The aim of the current study was, therefore, to provide evidence that might point to amylin and amylin-(8–37) having significant but opposing effects on lipid metabolism in vivo and to define the nature of the regulation.

RESEARCH DESIGN AND METHODS

Animal care and maintenance. All experiments were approved by the Animal Experimentation Ethics Committee of Garvan Institute of Medical Research/St. Vincent’s Hospital under the guidelines of the National Health and Medical Research Council of Australia. Male Wistar rats (250–300 g) were used for the study. The animals were kept at 22 ± 1°C with a 12:12-h light-dark cycle (lights on at 0600) and fed a standard chow diet (Allied Feeds, Rhodes, Australia).

Experimental procedures. A week before experiment, the left carotid artery and right jugular vein were cannulated with ketamine/xylazine (90 mg/kg) anesthesia. The cannulation procedure involved ligations of distal ends of both vessels and catheter insertions toward the heart to connect both sampling and administration lines directly to the systemic circulation. Rats were housed individually with food intake and body weight closely monitored and were handled daily to minimize stress. On the experiment day, food was removed at 0600, and the cannulas were connected to infusion apparatus (via the carotid line) and a blood-sampling syringe (via the jugular line) between 0830 and 0930. The experiment was performed under conscious conditions with free access to water. After a period of settlement (50–60 min), two basal samples (0.4 and 0.25 ml) were collected at (50–60 min), two basal samples (0.4 and 0.25 ml) were collected at 30, 60, 90, and 120 min, within which the accumulation of triglyceride content.

Metabolite measurements. Plasma glucose and lactate were measured by a glucose-lactate analyzer (YSI 2300, Yellow Springs, OH). Plasma NEFA was determined by spectrophotometer with the use of an acyl-CoA oxidase-based colorimetric kit (NEFA-C; WAKO Pure Chemical Industries, Osaka, Japan). Plasma triglycerides and glycerol were measured using enzymatic colorimetric methods (Triglyceride INT, procedure 336 and GPO Trinder, Sigma Diagnostics). The true plasma triglyceride was calculated by subtracting glycerol from the measured total triglyceride pool according to instructions of the kit. Plasma insulin was determined by RIA using a double-antibody method (Linco, St. Louis, MO). Tissue triglycerides were extracted with the method of Bligh and Dyer (4) and were measured by a Peridochrom Triglyceride GPO-PAP kit (Boehringer Mannheim, Mannheim, Germany). Plasma amylin concentrations were determined by RIA as described previously (15).

Statistics. All results are presented as means ± SE. A two-way ANOVA with time intervals as repeated measures was applied for statistical analyses. When necessary, a one-way ANOVA fractional comparison followed by post hoc tests (Fisher’s protected least significant difference test) was used to assess differences between groups at a particular time point. All statistical analyses were performed using Minitab Statview SE+Graphical program (Abacus Concepts-Brain Power). A value of P < 0.05 was considered to be statistically significant.
AMYLIN, LIPIDS, AND INSULIN RESISTANCE

Table 1. Preinfusion basal plasma parameters of the experimental groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline</th>
<th>Amylin</th>
<th>Amylin-(8–37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>7.6 ± 0.2</td>
<td>7.9 ± 0.3</td>
<td>7.3 ± 0.3</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>0.75 ± 0.08</td>
<td>0.66 ± 0.07</td>
<td>0.66 ± 0.03</td>
</tr>
<tr>
<td>NEFA, mmol/l</td>
<td>0.71 ± 0.05</td>
<td>0.63 ± 0.04</td>
<td>0.72 ± 0.05</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>0.37 ± 0.09</td>
<td>0.33 ± 0.03</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>Insulin, mU/l</td>
<td>38.2 ± 3.8</td>
<td>37.4 ± 3.3</td>
<td>39.7 ± 3.2</td>
</tr>
</tbody>
</table>

Values are means ± SE of 30- and 0-min samples; n = 6–8 rats per group. NEFA, nonesterified fatty acids.

RESULTS

Effects of amylin and amylin-(8–37) on plasma glucose, lactate, insulin, and lipids. The preinfusion basal values of plasma lipids, glucose, lactate, and insulin were similar among the groups (Table 1). Amylin infusion moderately increased plasma glucose (P < 0.01 vs. control, ANOVA with repeated measures) with a peak 13% increase above basal at 30 min and remained significantly higher at 240 min with a small decline (Fig. 1). Plasma lactate was elevated (P < 0.01 vs. control), largely contributed by increases between 30 and 60 min. The overall plasma insulin levels were higher than those of control (P < 0.05 vs. control), particularly between 30 and 120 min (P < 0.05). Amylin substantially elevated circulating NEFA and glycerol levels (both P < 0.001 vs. controls; Fig. 1). Both elevations were gradual but well sustained, with maximal increases of 80% above their basal values between 120 and 240 min.

Amylin-(8–37) infusion had opposite effects to those of amylin infusion. There were decreases in plasma glucose (P < 0.05 at 120 and 180 min), insulin (P < 0.05 at 60 and 120 min), NEFA (P < 0.05 at 60 and 180 min), and glycerol (P < 0.05 at 240 min) compared with the saline controls (Fig. 1). Further comparisons of the changes over the basal values between control and amylin-(8–37) groups with a two-way ANOVA with time as a repeated measure showed that plasma levels of glucose, insulin, NEFA, and glycerol were significantly decreased by amylin-(8–37; P < 0.05 vs. saline-infused group) with maximal decreases of 8, 40, 22, and 28% below their basal values, respectively. A similar lipid-lowering effect was also observed with amylin-(8–37) at a 10-times-less infusion rate (12.5 nmol/h; data not shown). However, plasma triglyceride levels were not significantly altered by either amylin or amylin-(8–37) with any statistical analyses.

Effects of amylin and amylin-(8–37) on liver and muscle tissue triglyceride content. Figure 2 shows triglyceride content in liver and red quadriceps muscle at the end of 4-h infusion. Liver triglyceride content was 28% higher in the amylin-infused group compared with control (6.44 ± 0.19 vs. 5.03 ± 0.18 μmol/g, P < 0.001), and there appeared to be a tendency toward elevated muscle triglycerides (3.29 ± 0.24 vs. 2.79 ± 0.18 μmol/g, P = 0.11). Triglyceride content in the liver and muscle was not significantly changed by amylin-(8–37) infusion.

Effects of amylin-(8–37) on amylin-mediated changes in lipids, glucose, and insulin. Figure 3 shows the effect of amylin-(8–37) on amylin action to alter plasma glucose, insulin, NEFA, and glycerol levels. Their basal values (7.8 ± 0.1 mmol/l, 38.8 ± 4.1 mU/l, and 0.71 ± 0.07 and 0.36 ± 0.04 mmol/l, respectively, n = 6) were similar to those of control and amylin-infused groups. In the presence of this antagonist, amylin’s effects on plasma glucose and insulin were completely eliminated (both P < 0.005 vs. amylin alone). There was also an overall blocking effect on amylin-induced changes in plasma NEFA and glycerol (both P < 0.005 vs. amylin alone). There were no significant differences in these parameters between the amylin-(8–37) plus amylin group and the saline infusion group.

Effects of acipimox on amylin-induced changes. Because amylin induced a lipolytic-like response (Fig. 1), we used the anti-lipolytic agent acipimox to examine
whether increased lipolysis was responsible for amylin’s action on lipid metabolism. As illustrated in Fig. 4, acipimox lowered plasma NEFA and glycerol for rats infused with both saline and amylin (P < 0.01 vs. their corresponding controls and basal values as shown at 0 min). It almost completely abolished the effect of amylin to elevate plasma NEFA and glycerol (P < 0.05 vs. amylin alone). After the preinjection of acipimox, there were no significant differences in plasma NEFA and glycerol levels before 180 min between saline control and amylin infusion (P > 0.05). However, amylin’s effect on plasma lactate levels was not altered by acipimox. Although amylin-induced increases in plasma glucose were not affected before 60 min, its levels were markedly decreased at 120 min and onward (P < 0.01 vs. amylin group, P > 0.05 vs. saline infusion group). Plasma insulin was lower in the amylin-infused group after acipimox injection (P < 0.05 vs. acipimox plus saline infusion), showing a response opposite to that induced by amylin alone (P < 0.01).

To examine whether acipimox could reverse amylin-induced NEFA and glycerol elevation, acipimox was administered after 120 min of amylin infusion and was monitored over the following 45 min. This period was chosen because amylin-elevated plasma glucose, NEFA, and glycerol levels were steady, and these parameters were similar to the basal values for the saline control group (see Fig. 1). As shown in Fig. 5, after acipimox injection, the elevated plasma NEFA and glycerol (P < 0.05 vs. preinfusion levels) were decreased to levels similar to those of acipimox-treated saline-infused controls (P > 0.05, ANOVA), whereas glucose remained moderately elevated (P < 0.05) at levels similar to those seen in the absence of acipimox.

Effect of amylin on hepatic triglyceride output rate. Figure 6 shows the effect of amylin on hepatic triglyceride output rate in 18-h-fasted rats by means of the Triton technique. Triton injection led to an almost linear accumulation of triglycerides in both groups. Compared with saline controls, amylin reduced plasma triglyceride concentrations after Triton injection (P < 0.001) within the period of 120 min, despite increases in plasma lactate levels (P < 0.001). The calculated hepatic triglyceride output rate was 0.88 ± 0.04 mmol·kg⁻¹·min⁻¹ with amylin infusion, significantly lower than that of control (1.48 ± 0.11 mmol·kg⁻¹·min⁻¹, P < 0.01). Amylin-(8–37) did not
show any significant effect on either hepatic triglyceride output rate or plasma lactate levels (data not shown).

**Plasma amylin levels.** Plasma levels of amylin were determined before and after amylin infusion in the group \((n = 6)\) that received amylin alone. Basal plasma levels were \(37 \pm 8\) and \(44 \pm 8\) pmol/l at 30 and 0 min, respectively, before infusion commenced, rising to \(1,140 \pm 280\) and \(1,040 \pm 270\) pmol/l after 1- and 4-h amylin infusion, respectively.

**DISCUSSION**

The present study shows for the first time that amylin infusion is able to increase plasma NEFA, which is paralleled by rises in plasma glycerol in a similar proportion. These concomitant elevations in plasma NEFA and glycerol mimicked a profile of increased lipolysis. Associated with increases in plasma NEFA, liver triglyceride content was also elevated. Data in this study also confirm that rat amylin-(8–37) is acting principally as an amylin antagonist (1, 2, 15, 34), and decreases in plasma NEFA, glycerol, and insulin levels were observed, opposite to the changes induced by amylin infusion. Furthermore, amylin-(8–37) blocked metabolic changes induced by infused amylin.

Effects of amylin on plasma glucose and lactate have been well characterized (5, 21, 28, 36) and were also observed in this study. Compared with responses in plasma glucose, lactate, and insulin, amylin-induced elevation in plasma NEFA and glycerol was slower in onset but more sustained. Because insulin can suppress plasma NEFA levels, the delayed increases in plasma NEFA and glycerol may be due to the higher plasma insulin levels driven by a larger increase in glucose at the early stage.

Until now, it has been a matter of debate as to what are the physiological or pathophysiological implications of experimentally demonstrable metabolic effects of exogenous amylin administration. Use of specific antagonists can point to possible effects of endogenous humoral factors. Thus the fact that amylin and amylin-(8–37) produce mutually reciprocal effects on lipid metabolism and that amylin-(8–37) can block amylin’s effects leaves open the possibility that endogenous amylin may have a role in modulating lipid metabolism under certain pathological conditions. This interpretation is consistent with a recent observation in goats that a sixfold increase in plasma amylin levels led to a 168% increase in plasma NEFA (24). In the present study, plasma amylin levels were \(~1,000\) pmol/l, approaching but still above the pathophysiological range in rats in our study. Plasma amylin concentrations have been reported to be as high as \(350\) pmol/l during growth hormone and dexamethasone treatment of mice (10) and \(600\) pmol/l in insulin-resistant and diabetic rodents (14). Therefore, further studies with reduced amylin infusion are required to establish the physiological implications of the present findings.

A mechanism for an amylin-induced increase in circulating lipids has not been described before. In general, increases in plasma lipids can be accounted for by either augmented appearance or, alternatively, by suppressed disappearance. Three major pathways are theoretically possible, leading to elevated plasma lipids: 1) an accelerated gut absorption, 2) enhanced hepatic output rate, particularly for triglycerides, or 3) lipoly-
sis of stored triglycerides. In terms of the possibility of enhanced absorption of lipids from the digestive system, high amylin affinity binding sites have been detected in the gastric fundus (3). However, amylin is known to inhibit gastric emptying and to delay gut 3-O-methyl-[3H]glucose absorption in rats (13). It appears unlikely, therefore, that amylin could exclusively enhance lipid absorption. Even if it did stimulate gut lipid absorption, triglyceride-rich chylomicrons should be the major lipid, and elevated plasma triglycerides would be apparent rather than elevated circulating NEFA. Our results of unaltered plasma triglycerides do not support such an interpretation.

As for the second possibility, Suzuki et al. (32) reported a direct stimulation by amylin of lipogenesis in primary cultured rat hepatocytes. However, it has been difficult to demonstrate convincingly reproducible effects of amylin on isolated rat hepatocytes (28). It has been proposed that, as well as enhancing the Cori cycle, amylin may indirectly promote hepatic lipogenesis by means of elevated plasma lactate as substrate (35). This hypothesis appears to be consistent with increased plasma lactate and liver triglyceride content. However, the results with Triton injection showed that amylin inhibited hepatic triglyceride output rate even though lactate was markedly increased. Although it could be argued that the increased liver triglyceride content might be attributable to an inhibition of hepatic triglyceride output rate, the reduced triglyceride appearance in plasma does not explain plasma NEFA and glycerol elevation. Furthermore, lactate infusion has no effect to increase plasma NEFA levels in vivo (22).

Theoretically, stimulation of cAMP could lead to activation of hormone-sensitive lipase (HSL) in adipocytes, a key enzyme in the conversion of stored triglycerides into NEFA and glycerol (16). The evidence to support this possibility comes from the present results showing a specific blockade by acipimox of amylin’s ability to increase plasma NEFA and glycerol and a complete reversal of amylin-elevated plasma NEFA and glycerol levels by this anti-lipolytic agent. Acipimox is a derivative of nicotinic acid. Its anti-lipolytic action is mediated through suppression of intracellular cAMP levels, with subsequent decrease in protein kinase A activity, leading to the reduced association of HSL with triglyceride substrate in the lipid droplet of adipocytes (6).

It is of interest to note that one of the major features of the amylin-elicited lipid changes is that elevations in plasma NEFA and glycerol levels occur with little change in plasma triglycerides, indicating a possible lipolytic action of amylin. Higher triglyceride content in the liver and possibly in muscle after amylin infusion might be due to increased plasma NEFA and glycerol from adipose tissue as substrates for triglyceride synthesis in these tissues. Indeed, amylin has been shown to stimulate the cAMP signaling pathway in various tissues (20, 28, 31). The involvement of the cAMP signaling pathway in amylin-mediated biological responses is also strongly supported by the recent discovery of amylin receptors, identified as receptor activity-modifying protein (RAMP) 1 and 3. Amylin stimulation of RAMP 1 and 3 is tightly coupled with cAMP accumulation, with EC₅₀ values between 0.1 and 1.87 nM (7).

Another possible interpretation for amylin-induced lipolysis would be that amylin acts by increasing sympathetic activity. Increased release of sympathetic hormones can also lead to lipolysis by β-adrenergic stimulation of the cAMP-protein kinase A-HSL pathway. A recent study in humans has shown similar responses of plasma glucose, insulin, lactate, and NEFA during an infusion of epinephrine for 120 min (19). In that study, the time-response pattern of these plasma metabolites was different from that induced by amylin in the present study in that plasma NEFA was rapidly but only temporarily increased, whereas increases in plasma glucose and lactate remained steadily elevated without any decline. Additionally, two separate earlier studies (18, 25) showed an inability of adrenergic antagonists to block amylin-induced metabolic changes. Therefore, it appears more likely that amylin may induce lipolysis by itself.

However, there is increasing evidence for a potent central anorectic effect of amylin (29). As well as anorectic effects, some findings, such as amylin knock-out mice gaining excess adiposity (12) and amylin-infused rats losing adiposity (29), could conceivably be related to a lipid-mobilizing action of amylin as suggested by our studies here, and further studies may clarify this.

The effect of amylin infusion on plasma insulin response is consistent with an indirect effect induced by circulating NEFA elevation. The crucial role of NEFA in insulin secretion in vivo has been previously illustrated by Dobbins et al. (11). They have demonstrated in the rat that lowering plasma NEFA levels by nicotinic acid suppresses plasma insulin elevation stimulated by all tested secretagogues, including glucose. In the present study, amylin infusion alone led to marked increases in plasma insulin, a phenomenon also reported by others (32). We suggest that amylin-induced increases in plasma insulin levels could be driven by combined increases in plasma glucose and NEFA, because amylin has been shown to inhibit glucose-stimulated insulin secretion in the isolated pancreas preparation in the absence of added fatty acids (1, 34). In support of this argument, our results also showed that amylin actually lowered plasma insulin levels despite the presence of hyperglycemia when NEFA levels were markedly decreased by acipimox. Hence, these results taken together indicate that, without the auxiliary effect of NEFA elevation, hyperglycemia alone is insufficient to overcome amylin’s inhibition of insulin secretion. The hypoinsulinemic response, along with the fact that amylin-induced increases in plasma glucose levels were inhibited in the later stage after the reduction in plasma NEFA, appears to suggest an important role of amylin-modulated lipid metabolism in insulin resistance caused by this hormone.
In summary, the present study examined the effects of amylin and amylin-(8–37) on lipid metabolism in relation to glucose metabolism. We have obtained evidence showing effects of amylin to increase plasma NEFA and glycerol in normal rats that adds to its well known actions on plasma glucose and insulin. Reciprocally, amylin-(8–37) lowers these parameters itself and blocks amylin-stimulated increases in plasma NEFA, glycerol, glucose, and insulin. Amylin-induced increases in plasma NEFA and glycerol as well as insulin, but not glucose and lactate, can be eliminated by a specific anti-lipolytic agent. These results indicate that amylin may have a lipolytic action in vivo that could also contribute to amylin-induced insulin resistance.

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