Rat amylin-(8—37) enhances insulin action and alters lipid metabolism in normal and insulin-resistant rats

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Hettiarachchi, M., S. Chalkley, S. M. Furler, Y.-S. Choong, M. Heller, G. J. S. Cooper, and E. W. Kraegen. Rat amylin-(8—37) enhances insulin action and alters lipid metabolism in normal and insulin-resistant rats. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E859–E867, 1997.—To clarify roles of amylin, we investigated metabolic responses to rat amylin-(8—37), a specific amylin antagonist, in normal and insulin-resistant, human growth hormone (hGH)-infused rats. Fasting conscious rats were infused with saline or hGH, insulin-resistant, human growth hormone; euglycemic clamp; muscle; liver; triglycerides; long-chain acyl-CoA

Amylin, a 37-amino acid polypeptide, was first isolated and characterized from islet amyloid deposits in patients with non-insulin-dependent diabetes mellitus (NIDDM) (7). Immunohistochemical studies have revealed that amylin is cosecreted with insulin from pancreatic β-cells (24). Despite considerable investigation, the metabolic role of amylin is far from clear (7). Amylin has the potential to exert an autocrine or paracrine effect on islet insulin secretion (33), and in addition a number of extrapancreatic actions of amylin have been reported, although controversy remains as to their physiological importance. In muscle, amylin has been shown to cause glycogenolysis and oppose glycogen synthesis (8) and, as a result, increases lactate levels in plasma (38). Amylin administration also results in increased hepatic glucose production (16, 23).

Amylin has been implicated as a factor in insulin-resistant states (18). Evidence of increased circulating levels has been found in states such as obesity and NIDDM (7), suggesting that hyperamylinemia may accompany the hyperinsulinemia, which is a characteristic of such states.

We recently demonstrated that continuous infusion of human growth hormone (hGH) causes prompt hyperinsulinemia and insulin resistance in the rat (14). Here we reasoned that hGH-induced hyperinsulinemia may be accompanied by hyperamylinemia and that this may contribute to the generation of hGH-induced insulin resistance. Another factor that may contribute to the onset of hGH-induced (9, 14) and diet-induced (31) insulin resistance is increased systemic and muscle lipid availability. However, except for two negative reports (8, 21), related to adipose tissue lipolysis, we are unaware of any studies examining whether there is any link between amylin and lipid metabolism.

In the past, it has often proved difficult to demonstrate the physiological effects of an endogenous hormone by administration to biological systems of small quantities of the hormone itself (sufficient only to produce near-physiological increments in concentration). Specific antagonists of hormone action have often proved to be more reliable indicators of the physiological role played by an endogenous hormone (4). Here we have used the principle of syntopic antagonism to probe the physiological role of endogenous amylin on fuel metabolism in the rat (4). The specific amylin antagonist amylin-(8—37) has previously been used to examine roles of amylin (2, 10), and we have employed this antagonist in our own study. Amylin-(8—37) is a truncated peptide of native rat amylin (35). In vivo (2) and in vitro (1, 3, 10, 35) studies suggest that it acts as a specific amylin antagonist. Further data related to the specificity of the action of amylin-(8—37) in muscle were obtained in the present study.

The present study was designed to assess the effects of amylin-(8—37) on glucose and lipid metabolism in normal and insulin-resistant rats under insulin-stimulated and basal conditions. The studies demonstrate profound effects favoring enhanced insulin action with concomitant alteration of lipid metabolism in both normal and insulin-resistant rats.

MATERIALS AND METHODS

Materials for Infusion

Rat amylin-(8—37) was obtained from Bachem (Torrance, CA; batch ZM554). Before use, it was verified that the...
molecular mass was exact by matrix-assisted laser desorption ionization mass spectrometry (Instrument G2025A MALDI-TOF, Hewlett-Packard). hGH for infusion was human recombinant growth hormone (potency 2.6 U/mg) produced from a mammalian cell line as previously described (15). For clamp periods, porcine insulin (Actrapid, Novo-Nordisk, Bagsvaerd, Denmark) was infused.

Animals

Ethical aspects. All surgical and in vivo experimental procedures performed were approved by the Garvan Institute of Medical Research-St. Vincent's Hospital Animal Experimentation Ethics Committee and were in accordance with the National Health and Medical Research Council of Australia guidelines for the use of animals in research. All in vitro muscle experiments were performed according to protocols approved by the University of Auckland Animal Ethics Committee.

In vivo animal preparation. Adult male Wistar rats (365 ± 8 g) were fed standard laboratory diet (Allied Feeds, Rhodes, NSW, Australia) with water ad libitum and were housed individually after surgery in enclosed, well-ventilated metabolic cages in a temperature controlled environment (22 ± 1°C) with 12:12 h light-dark cycle (lights on at 0600). Rats were accustomed to human contact to minimize stress associated with handling during studies.

Surgery

One week before the day of the clamp, rats were chronically cannulated via the right jugular vein and left carotid artery under ketamine hydrochloride (90 mg/kg)-xylazine (10 mg/kg) anesthesia. Cannulas were exteriorized, and patency was maintained as previously described (17). Subsequent recovery over a 7-day period was closely monitored with measurement of food intake and body weight gain.

Study Protocols

Infusion studies. Cannulated rats were randomly assigned to one of four infusion groups, continuously receiving saline (control), saline plus amylin-(8–37), hGH, or hGH plus amylin-(8–37) over a 5.75-h period. hGH (21 µg·kg⁻¹·h⁻¹) and amylin-(8–37) (0.125 µmol/h) were infused continuously in 0.9% saline (vol 600 µl/h) via the carotid cannula using a syringe pump (Harvard Instruments, S. Natick, MA). The in vivo amylin-(8–37) infusion rate was the same as previously used (2).

During the last 2 h of the infusions, insulin action was assessed using a euglycemic hyperinsulenic clamp as previously described (17). In brief, insulin was infused via the carotid cannula at a constant rate of 1.8 nmol·kg⁻¹·h⁻¹ (0.25 U·kg⁻¹·h⁻¹). The jugular cannula was used for sampling and tracer bolus administration. Euglycemia was maintained by a variable infusion of 30% wt/vol glucose. At 45 min before the end of the clamp, in a bolus dose of D-[U-¹⁴C]glucose was administered (3.4 and 6.8 MBq/kg, respectively). At 45 min before the end of the infusion period, the rats were killed with a lethal dose of pentobarbital sodium (150 mg/kg, Nembutal, Abbott Laboratories, Sydney, Australia). Soleus, red gastrocnemius, white gastrocnemius, red quadriceps, and white quadriceps muscle, white adipose tissue (epididymal fat pads), liver, heart, and interscapular brown adipose tissue were rapidly removed, freeze-clamped with liquid nitrogen, and then stored at −70°C for subsequent assays. Hematocrit remained above 40% in all studies.

Extended basal studies. Additional saline and saline plus amylin-(8–37) groups were used for longer basal studies, extended for the whole 5.75-h infusion period. Plasma samples were collected hourly. After collection and plasma separation, erythrocytes were resuspended in saline and injected back into the rat. Forty-five minutes before the end of the infusion, a bolus dose of tracer was administered (as for clamp protocols), and plasma and tissue samples were collected and processed as described above.

Estimation of individual muscle glucose metabolic index. Frozen tissues (50–100 mg) were homogenized in 10–20 vol of ice-cold water for 30 s using an Ultra-Turrax (IKA, Seefeld, Germany). With 0.2-ml supernatant samples, free and phosphorylated 2-[³H]DG were separated by ion-exchange chromatography on Dowex 1-X8 (100–200 mesh, acetate form, Bio-Rad, Richmond, CA) (17). A further 0.2 ml of the supernatant was counted directly to provide the total tracer content. The accumulation of tissue phosphorylated 2-[³H]DG and the plasma 2-[³H]DG disappearance curve were used to calculate the glucose metabolic index (R d), an estimate of the tissue-specific glucose uptake rate (17). Other analyses for tissue glycogen and triglyceride content were performed as described previously (5, 13).

Actions of amylin and amylin-(8–37) in isolated incubated soleus muscle strips. Male Wistar rats weighing 148 ± 11 g were anesthetized with pentobarbital sodium (45–50 mg/kg body wt) and then killed by cervical dislocation. Soleus muscles were quickly dissected out under carbon dioxide Krebs-Henseleit buffer (KHB) and teased into two halves. After a further washing step in KHB and a preincubation for 30 min in Dulbecco’s modified Eagle’s medium containing 0.1% (wt/vol) bovine serum albumin and no added peptides, insulin, or insulin with increasing amounts of peptides, respectively, 0.5 µCi D-[U-¹³C]glucose was added, and the muscle strips were incubated for another 2 h at 37°C under a constant gas flow of 95% O₂-5% CO₂ in a shaking water bath. Thereafter the strips were blotted dry on filter paper, the tendons were excised, and strips were snap frozen in liquid nitrogen. The frozen muscle strips were then freeze dried. About 5 mg of freeze-dried muscle were solubilized in 0.4 ml 60% (wt/vol) KOH for glycogen analysis. The glycogen was precipitated overnight at −20°C with 1.2 ml absolute ethanol, centrifuged (8,000 g, 15 min, 4°C), and washed twice with 1.2 ml ice-cold ethanol. The dried glycogen precipitate was then dissolved in 0.6 ml of water. One aliquot (0.3 ml) was used to measure [¹³C]glycogen formation by liquid scintillation spectrometry as a measure of de novo glycogen synthesis, and the other was hydrolyzed to D-glucose by adding 1 U of glucan 1,4-α-glucosidase enzyme activity from Aspergillus niger (Sigma, St. Louis, MO) and making up the volume to 0.5 ml with 0.2 M sodium acetate (pH 4.8). The glucose concentration was analyzed with an YSI 2300 Stat glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). The total glycogen content in each muscle strip is expressed as micromoles of glucosyl units per gram of dry weight.
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Other analytical methods for in vivo studies. Blood and plasma glucose concentrations were measured using a YSI 23AM glucose analyzer. Plasma nonesterified fatty acids (NEFA) were measured by an acyl-CoA oxidase-based colorimetric assay method (Wako Pure Chemical Industries, Osaka, Japan), and plasma triglycerides were estimated using a colorimetric assay (Triglyceride Procedure 336, Sigma Diagnostics). Plasma glycerol was measured using a colorimetric assay (GPO Trinder) from Sigma Diagnostics. Plasma insulin was measured using a double-antibody radioimmunoassay (RIA) kit (Linco, St. Louis, MO). Plasma hGH was measured as previously described (36), using an in-house RIA with an antiserum against pituitary-derived hGH raised in rabbits and a double-antibody polyethylene glycol-facilitated precipitation technique.

Tissue long-chain acyl-CoA measurement. The method for measurement of total tissue long-chain acyl-CoA is as previously described (14). In brief, the methodology involves solvent extraction of long-chain acyl-CoA from tissues, phase separation and purification by column elution, and injection of the samples onto a Novapak C18 reverse-phase high-performance liquid chromatography (HPLC) column (Waters Millipore) for ultraviolet detection. The identification of each species was from its respective retention times obtained by applying a known amount of each species to the HPLC column before analysis of the samples. The reported parameter is the sum of the six major species determined [palmitoyl (16:0)-, palmitoleoyl (16:1)-, linolenoyl (18:3)-, linoleoyl (18:2)-, oleoyl (18:1)-, and stearoyl (18:0)-CoA].

Plasma amylin assay. Plasma amylin was extracted using an acetone-HCl (1 M)-water mixture (40:1:5; vol/vol/vol) with a ratio of 1:2 (wt/vol) for plasma to extraction solution (28). This gave a measured optimal recovery of 58% in our hands. All plasma amylin concentrations were corrected for the incomplete extraction using a multiplier of 1.0/0.58. Supernatants were lyophilized and stored at −80°C until analysis. Peptide concentrations after reconstitution in assay buffer were determined in duplicate using a commercial RIA kit (Peninsular Laboratories, Belmont, CA). Rat amylin-(8—37) gave <5% cross-reactivity (nonparallel) with the rat amylin standard; for this reason, endogenous amylin could not be measured in samples from rats infused with amylin-(8—37). The limit of detection for plasma amylin was 3 pM, the linear range was 5–125 pM, and the intra-assay coefficient of variation was <3% at the midrange.

Statistical Analysis

All results are expressed as means ± SE. Statistical comparisons between control and hGH-treated groups were performed using two-factor analysis of variance and the Student’s unpaired (2 tail) t-test (Macintosh Statview SE + Graphics program, Abacus Concepts-Brain Power).

RESULTS

In Vitro Studies of Amylin-(8—37)

Specificity in Muscle

Amylin-(8—37) in absence of amylin. Initial in vitro studies in isolated incubated soleus muscle strips demonstrated that 10 µM amylin-(8—37) had no effect in the absence of amylin on either the basal or insulin-stimulated net rate of [14C]glucose incorporation into glycogen (Fig. 1). In contrast, 30 nM amylin significantly decreased insulin-stimulated [14C]glucose incorporation into glycogen (Fig. 1).

Statistical analysis performed using two-factor analysis of variance and the comparisons between control and hGH-treated groups were statistically significant.

Fig. 1. Effects of amylin and amylin-(8—37) on control (no insulin) and insulin-stimulated D-[U-14C]glucose incorporation into glycogen in isolated soleus (Sol) muscle strips incubated for 2 h. Incubation medium contained saline, 10 µM amylin-(8—37), 23.7 nM insulin, 23.7 nM insulin + 10 µM amylin-(8—37), and 23.7 nM insulin + 30 nM amylin as indicated. Results are expressed as means ± SE for n = 6–12. *P < 0.05 vs. 23.7 nM insulin.

Amylin-(8—37) inhibition of amylin action. As shown in Fig. 2, 10 nM amylin significantly decreased net insulin-stimulated [14C]glucose incorporation rate into glycogen. This inhibitory effect was reversed by addition of 10 µM amylin-(8—37) to the incubation medium. Final glycogen mass among groups (Fig. 2) showed a similar pattern to the [14C]glucose incorporation measurements but was not significantly different among groups.

In Vivo Studies

Predexamethasone responses. Plasma amylin levels after 2 or 3.75 h of hGH infusion were increased approximately threefold compared with saline-infused rats (P < 0.02) but were not significantly different from saline controls at the end of the glucose clamp (Table 1). Amylin concentrations were determined only for the saline- and hGH-infused groups, since amylin-(8—37) cross-reacted in the RIA for amylin. During predexamethasone infusions, plasma glucose levels were similar at ~7 mM in all the groups (Table 2). Infusion of hGH alone raised plasma insulin and NEFA levels, but when combined with amylin-(8—37) infusions, insulin and NEFA remained at saline-infused levels. Amylin-(8—37) infusion also suppressed plasma insulin and NEFA levels in the saline plus amylin-(8—37) group compared with the saline control (Table 2). However plasma triglycerides were significantly elevated by amylin-(8—37) in both treated groups (Table 2).

Clamp responses. Glucose clamps were performed at similar plasma glucose levels in all four groups (Table 3). Clamp insulin levels were also similar in the four
groups (Table 3). Plasma triglycerides were suppressed to equivalent levels in all groups during the clamp. Infusion of hGH for 5.75 h caused peripheral insulin resistance similar to that shown previously (14), characterized by a decreased clamp glucose infusion rate (GIR) and \( R_d \) compared with the saline control (Table 3). Furthermore, elevated HGO during the clamp indicated impaired insulin suppressibility of HGO in the hGH-infused group. Infusion of amylin-(8—37) prevented hGH-induced insulin resistance (i.e., GIR, \( R_d \), and HGO were not different from saline-infused levels). Furthermore, amylin-(8—37) significantly increased insulin-stimulated \( R_d \) in the saline-infused plus amylin-(8—37) group (Table 3).

Clamp \( R_d \). As shown in Fig. 3, insulin-stimulated red quadriceps \( R_d \) was significantly enhanced by amylin-(8—37), both in the hGH-infused and saline control groups. Similar significant effects were also found in red gastrocnemius muscle (saline 15.8 ± 1.8, saline + amylin-(8—37), 20.8 ± 1.5 (\( P < 0.05 \) vs. saline), hGH 8.9 ± 1.6, and hGH + amylin-(8—37) 15.0 ± 1.2 \( \mu \text{mol·100 g}^{-1} \cdot \text{min}^{-1} \) (\( P < 0.01 \) vs. hGH)). This is consistent with the enhancement of whole body insulin sensitivity parameters GIR and \( R_d \) (Table 3). The insulin-stimulated \( R_d \) of white adipose tissue was also significantly enhanced by amylin-(8—37) infusion in the hGH-treated group but not in the saline control group (Fig. 3).

Insulin-stimulated glycogen synthesis. Amylin-(8—37) infusion significantly increased insulin-stimulated \([14C]\)glucose incorporation into glycogen in red gastrocnemius muscle in both hGH- and saline-treated groups (Fig. 4). Glycogen content at the end of the clamp (Fig. 4) followed a similar pattern. The in vivo findings are consistent with the in vitro responses in soleus muscle strips (Figs. 1 and 2).

Extended basal responses. Because it appeared that there were significant effects of amylin-(8—37) in saline-infused rats, further studies were performed to examine this in more detail. In these studies, infusion of saline or saline plus amylin-(8—37) was performed for 5.75 h without a hyperinsulinemic clamp. Plasma glucose remained at ~7 mM in both groups. With amylin-(8—37) infusion, plasma insulin, NEFA, and glycerol decreased, whereas plasma triglycerides increased (Table 4), each consistent with results presented in Table 2. Plasma glycerol was additionally measured and was significantly decreased compared with controls, suggesting that decreased lipolysis is a contributor to the lowered NEFA levels (Table 4). Basal glucose turnover (HGO and \( R_d \)) was significantly
reduced in the saline plus amylin-(8—37) group compared with saline alone (Table 4). Red muscle $R_g$ measured under basal conditions was not altered by amylin-(8—37) [saline vs. saline + amylin-(8—37), 19.4 ± 1.4 vs. 17.5 ± 1.4 µmol·100 g$^{-1}$·min$^{-1}$].

Muscle and liver lipids. As shown in Fig. 5, amylin-(8—37) infusion resulted in elevated liver triglyceride content ($P < 0.05$ vs. control), whereas red muscle triglyceride content was significantly reduced ($P < 0.05$ vs. control). Total long-chain acyl-CoA (sum of all species) levels in liver and red muscle were increased and decreased respectively, by amylin-(8—37) infusion,

Table 3. Influence of amylin-(8—37) infusion on hyperinsulinemic euglycemic clamp responses during saline or hGH infusion

<table>
<thead>
<tr>
<th></th>
<th>hGH Infusion</th>
<th>hGH + Amylin-(8—37) Infusion</th>
<th>Saline Infusion</th>
<th>Saline + Amylin-(8—37) Infusion</th>
<th>Significance of hGH Effect</th>
<th>Significance of Amylin-(8—37) Effect</th>
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<tbody>
<tr>
<td>Plasma parameters</td>
<td></td>
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<tr>
<td>Glucose, mM</td>
<td>6.9 ± 0.1</td>
<td>7.0 ± 0.1</td>
<td>7.1 ± 0.1</td>
<td>6.9 ± 0.3</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Insulin, mU/l</td>
<td>105 ± 8</td>
<td>107 ± 6</td>
<td>119 ± 21</td>
<td>98 ± 5</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Triglycerides, mM</td>
<td>0.67 ± 0.1</td>
<td>0.78 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.62 ± 0.1</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>NEFA, mM</td>
<td>0.41 ± 0.1</td>
<td>0.50 ± 0.1</td>
<td>0.62 ± 0.1</td>
<td>0.64 ± 0.1</td>
<td>NS</td>
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<tr>
<td>Glucose fluxes</td>
<td></td>
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<tr>
<td>GIR, mg·kg$^{-1}$·min$^{-1}$</td>
<td>18.0 ± 0.5</td>
<td>23.7 ± 0.8</td>
<td>25.2 ± 1.0</td>
<td>29.5 ± 1.5</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>$R_d$, mg·kg$^{-1}$·min$^{-1}$</td>
<td>22.7 ± 0.8</td>
<td>25.3 ± 0.9</td>
<td>24.6 ± 0.7</td>
<td>27.9 ± 1.0</td>
<td>$P &lt; 0.02$</td>
<td>$P &lt; 0.002$</td>
</tr>
<tr>
<td>HGO, mg·kg$^{-1}$·min$^{-1}$</td>
<td>4.2 ± 0.9</td>
<td>0.8 ± 1.2</td>
<td>−1.6 ± 0.6</td>
<td>−1.4 ± 1.6</td>
<td>$P &lt; 0.0005$</td>
<td>NS</td>
</tr>
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Values are means ± SE; $n = 6–11$. GIR, glucose infusion rates; $R_d$, glucose disposal rate; HGO, hepatic glucose output. Significance analysis by 2-factor ANOVA.

![Fig. 3. Insulin-stimulated glucose metabolic index ($R_g$) in red quadriceps (A) and white adipose tissue (B) during hyperinsulinemic euglycemic clamps performed in saline- or hGH-infused rats with (+) or without (−) amylin-(8—37) infusion. Results are expressed as means ± SE for $n = 7–9$. *$P < 0.02$ vs. hGH without amylin-(8—37); †$P < 0.01$ vs. saline without amylin-(8—37).](http://ajpendo.physiology.org/)

![Fig. 4. Insulin-stimulated [$^{14}$C]glucose incorporation into glycogen (A) and glycogen mass (B) in red gastrocnemius muscle during clamps performed in saline- or hGH-infused rats with (+) or without (−) amylin-(8—37) infusion. Results are expressed as means ± SE for $n = 7–9$. *$P < 0.05$ vs. hGH; †$P < 0.05$ vs. saline.](http://ajpendo.physiology.org/)
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Table 4. Extended basal study: influence of 5.75 h of amylin-(8—37) plus saline infusion on plasma responses and basal glucose turnover (Rd/HGO)

<table>
<thead>
<tr>
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<th>Saline</th>
<th>Saline + Amylin-(8—37)</th>
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<tbody>
<tr>
<td>Plasma glucose, mM</td>
<td>7.5 ± 0.2</td>
<td>7.2 ± 0.2</td>
</tr>
<tr>
<td>Plasma insulin, mU/l</td>
<td>38.6 ± 2.4</td>
<td>28.1 ± 3.5*</td>
</tr>
<tr>
<td>Plasma triglycerides, mM</td>
<td>1.2 ± 0.1</td>
<td>1.8 ± 0.2*</td>
</tr>
<tr>
<td>Plasma NEFA, mM</td>
<td>1.2 ± 0.14</td>
<td>0.79 ± 0.06*</td>
</tr>
<tr>
<td>Plasma glycerol, mM</td>
<td>0.76 ± 0.2</td>
<td>0.28 ± 0.06*</td>
</tr>
<tr>
<td>Basal Rd/HGO, mg·kg⁻¹·min⁻¹</td>
<td>9.3 ± 0.6</td>
<td>7.5 ± 0.4*</td>
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</table>

Values are means ± SE; n = 6–11. *P < 0.05 vs. saline control.

reflecting the pattern in liver and muscle triglyceride measurements (Fig. 5).

DISCUSSION

Hyperamylinemia is usually present in insulin-resistant mammals (reviewed in Ref. 7). However, although high concentrations of exogenously administered amylin evoke insulin resistance in vivo and in incubated skeletal muscle strips in vitro, the contribution to in vivo insulin resistance made by increased circulating concentrations of endogenously derived amylin remains unknown. To investigate this relationship, secretion and action of amylin were studied in an animal model of insulin resistance, the hGH-infused rat (14). Amylin action was probed by infusion of the competitive amylin antagonist, amylin-(8—37), a synthetic peptide whose structure was derived by truncating the seven NH₂-terminal residues from rat amylin. Here, we report for the first time that infusion of hGH into rats evoked hyperamylinemia. Second, infusion of rat amylin-(8—37) into normal rats or those with hGH-evoked insulin resistance led to increased whole body insulin sensitivity. After treatment with amylin-(8—37), basal insulin concentrations in both groups were significantly decreased compared with those in the respective control groups. Furthermore, the extent of the fall in plasma insulin concentration was consistent with the increase in insulin sensitivity in both normal and insulin-resistant rats. Third, infusion of amylin-(8—37) elicited changes in circulating and tissue lipids in both normal and insulin-resistant rats. Of particular note is our observation that the fall in plasma NEFA concentrations elicited by amylin-(8—37) was the opposite of what could have been predicted on the basis of decreased circulating insulin concentrations. We know of no prior reports in the literature demonstrating effects of an amylin antagonist on tissue lipid distribution.

In the current study, we confirmed that amylin-(8—37) acts as an antagonist of the in vitro effects of amylin in skeletal muscle. In the absence of amylin, amylin-(8—37) altered neither basal nor insulin-stimulated rates of incorporation of D-[U-14C]glucose into glycogen. However, in the presence of amylin, it dose dependently reversed amylin-evoked inhibition of insulin-stimulated incorporation of D-[U-14C]glucose into glycogen. These findings are consistent with the previous report of Deems et al. (10). There are further lines of evidence to support the view that amylin-(8—37) acts as an antagonist at amylin receptors. In one study it completely displaced bound ¹²⁵I-labeled amylin from lung membranes (3); in another, it reversed amylin-mediated inhibition of secretion of insulin from rat pancreatic islets (35). The neuropeptide calcitonin gene-related peptide (CGRP) is structurally similar to amylin and mimics its effects in many biological systems (reviewed in Ref. 7). The truncated peptide CGRP-(8—37) also acts as an amylin blocker in some systems (11, 34). In a recent study, CGRP-(8—37), but not amylin-(8—37), inhibited CGRP-induced vasodilation in rat kidney (6), indicating that the former is specific for CGRP receptors in renal tissues. These results, when taken together, indicate that the peptide amylin-(8—37) is selective for amylin receptors.

In our current in vivo studies, amylin-(8—37) not only reversed effects evoked by hGH on carbohydrate and lipid metabolism but also further enhanced the insulin sensitivity of normal rats. This effect on insulin sensitivity in saline-treated rats indicates that this amylin blocker either inhibits the action of basal amylin present under nonstimulated conditions or that it can act independently of amylin receptors to enhance whole body insulin sensitivity. The latter notion, how-
However, is not supported by the in vitro findings in isolated incubated soleus muscle. Another possible mechanism for the enhancement of insulin sensitivity could be via enhanced blood flow. Because amylin and CGRP are structurally similar, CGRP is a potent vasodilator, and amylin is likely to act at CGRP receptors (7), it is conceivable that, by blocking the amylin acting on CGRP receptors, amylin-(8—37) might increase blood flow and thereby enhance insulin-induced Rg. However, this explanation cannot account for the action of amylin-(8—37) to restore insulin sensitivity in soleus muscle incubated in vitro. Furthermore, the findings of Gardiner et al. (11) show that amylin can evoke insulin resistance in vivo in the absence of hemodynamic effects.

Our finding that plasma insulin concentrations decrease after infusion of amylin-(8—37) is at possible variance with the findings of Wang et al. (35). However, a quite different experimental design was used, and the concentrations of amylin-(8—37) at the level of the pancreatic islet in their in vitro study were very much higher than could be expected in our current in vivo studies. There is evidence that amylin-(8—37) not only antagonizes amylin-evoked inhibition of insulin secretion (27) but also is capable of increasing insulin secretion when added by itself (in absence of amylin) to islets or to the isolated, perfused pancreas. (It should be noted, however, that these preparations secrete endogenous amylin.) In the light of these in vitro findings, we suggest that the decrease in plasma insulin without an observable change in plasma glucose concentrations, which we found in our current studies, is likely to be a response to the extrapancreatic actions of amylin-(8—37). The fact that altered basal insulin secretion and peripheral insulin sensitivity were tightly coupled is demonstrated in Fig. 6. The highly significant negative correlation (r = 0.70, P < 0.0001) between clamp GIRs and basal (preclamp) plasma insulin concentrations, derived from our data for all groups, provides further evidence for a direct relationship between increased insulin sensitivity and decreased insulin secretion. The identity of the metabolic signals involved is, however, currently uncertain. It has been suggested that circulating NEFA (32) or pancreatic β-cell long-chain acyl-CoA (29) could play important roles in determining the rate of insulin secretion. It is thus possible that the fall in insulin secretion is mediated by the fall in plasma NEFA, and we believe a similar situation may be operative after administration of the insulin-sensitizer thiazolidinedione, BRL-49653, which can reduce NEFA levels and basal plasma insulin levels without a demonstrable change in plasma glucose levels in rats (26). Alternatively, it could be argued that the decreases in plasma insulin that we observed here could be due to amylin-(8—37) acting as a partial agonist of amylin on insulin secretion. We believe this is unlikely, however, since there is no published evidence to indicate that amylin-(8—37) can act as a partial amylin agonist in any tissue, and here amylin-(8—37) clearly acted as an antagonist in muscle.

Infusion of amylin-(8—37) enhanced whole body as well as peripheral insulin sensitivity, as indicated by the increased clamp GIR and Rg in both hGH- and saline-treated groups. In addition amylin-(8—37) enhanced insulin suppressibility of HGO in hGH-infused rats. In line with the enhancement of peripheral insulin sensitivity by amylin-(8—37) infusion, muscle and white adipose tissue insulin-mediated glucose uptake was enhanced, as indicated by Rr. Significant enhancement of net glycojen synthesis, as measured by [14C]glucose incorporation into glycogen during the clamp procedure in amylin-(8—37)-infused rats, provides further evidence for improved peripheral insulin sensitivity. It is well documented that amylin has antagonistic effects on net glycogen synthesis in vivo (19, 39) and thus enhanced insulin-mediated net glycogen synthesis in the saline plus amylin-(8—37)-infused group is in accord with a blockade of endogenous amylin action. Regarding the liver, in other studies, it has been demonstrated that amylin is capable of stimulating lactate recycling in vivo (37). Therefore the improved insulin suppressibility of HGO in our study could be related to diminishing lactate recycling in the liver (direct Cori cycle).

It was interesting to find altered lipid distribution after amylin-(8—37) infusion, particularly in the group treated with saline plus amylin-(8—37) infusion, since there is little available evidence to suggest that amylin influences lipid metabolism (8, 21). In the present study, lowering of basal plasma NEFA and glycerol by amylin-(8—37) infusion in saline-treated animals is consistent with a decreased rate of lipolysis. The increase in plasma triglyceride concentration indicates increased production or decreased clearance of very low density lipoprotein-triglyceride or a combination of the two. Very low density lipoprotein production and secretion from the liver have been shown to be significantly affected by peripheral insulin concentrations (20), and the reduced plasma insulin levels in the current study after amylin-(8—37) infusion may be responsible for the increased plasma triglyceride concentrations. Alternatively, a decreased clearance of triglycerides into
skeletal muscle may simultaneously explain high plasma triglyceride levels and contribute to reduced muscle triglyceride and long-chain acyl-CoA levels. Studies of lipid turnover kinetics during amylin-(8–37) infusion are needed to resolve these issues. Increased liver triglycerides and long-chain acyl-CoA concentrations during amylin-(8–37) infusion indicate possible de novo triglyceride synthesis or recycling of NEFA.

The finding that in vivo amylin-(8–37) infusion leads to a significant reduction in muscle lipids adds a new dimension to possible mechanisms by which amylin may influence muscle insulin sensitivity. We have previously reported that there is a striking negative association between muscle insulin sensitivity and local triglyceride content in various rat diet models of altered muscle insulin sensitivity (31). In the case of hGH-induced insulin resistance, increased lipid mobilization from adipose tissue is a characteristic over the time frame studied here (14). The mechanisms by which altered muscle lipid availability could lead to altered potency of insulin action are currently not clear but could include the classic Randle cycle as well as the possibility of other mechanisms (22). In particular, a central role for accumulation of cytosolic long-chain acyl-CoA has been proposed (29); these could act as regulatory ligands for several enzymes of glucose metabolism (30). Alternatively altered muscle lipid availability may influence insulin signaling via diacylglycerol-protein kinase C interactions (25). Further studies are required to examine whether these interactions are important in the case of amylin (8–37) infusion and, if so, whether they are a direct or indirect effect within muscle.

Amylin-(8–37) reversed hGH-induced insulin resistance and hyperinsulinemia, suggesting a possible association between amylin and hGH-evoked insulin resistance. Hyperamylinemia was present throughout much of the initial phase of hGH infusion. However, that amylin concentrations had returned to normal by the end of the euglycemic clamp is consistent with a mechanism which altered muscle lipid availability could lead to altered potency of insulin action are currently not clear but could include the classic Randle cycle as well as the possibility of other mechanisms (22). In particular, a central role for accumulation of cytosolic long-chain acyl-CoA has been proposed (29); these could act as regulatory ligands for several enzymes of glucose metabolism (30). Alternatively altered muscle lipid availability may influence insulin signaling via diacylglycerol-protein kinase C interactions (25). Further studies are required to examine whether these interactions are important in the case of amylin (8–37) infusion and, if so, whether they are a direct or indirect effect within muscle.

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REFERENCES


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