Amylin-Mediated Reduction in Insulin Sensitivity Corresponds to Reduced Insulin Receptor Kinase Activity in the Rat In Vivo

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Studies were undertaken to elucidate further the mechanism whereby the pancreatic peptide amylin induces insulin resistance. Sixteen male Sprague-Dawley rats underwent hyperinsulinemic (14 pmol/kg/min, 0 to 120 minutes) euglycemic clamps in the presence or absence of amylin (500 pmol/kg/min, 60 to 120 minutes). Amylin induced insulin resistance at both the hepatic level (mean ± SE: hepatic glucose output [HGO] with amylin 1.4 ± 0.2 *v* without amylin −1.9 ± 0.3 mmol/kg/h, $P < .001$) and peripheral level (glucose disposal [R_d] with amylin 5.0 ± 0.2 *v* without amylin 8.5 ± 0.6 mmol/kg/h, $P < .001$). Serum insulin levels were similar in the presence or absence of amylin alone (661 ± 89 *v* 636 ± 50 pmol/L, respectively, P = NS), but were significantly less when somatostatin (SRIF) was simultaneously infused (408 ± 15 pmol/L, $P < .02$ *v* the other two groups). This suggests that endogenous insulin production was not suppressed by amylin under these study conditions. Similar findings were obtained in 18 animals in the absence of exogenous insulin infusion. In vitro kinase activity toward histone of skeletal muscle insulin receptors (IRs) activated by insulin in vivo was reduced in the presence of amylin to 6.0 ± 0.8 versus 9.1 ± 1.2 fmol phosphate into histone (insulin-infused) and 3.9 ± 0.7 versus 6.9 ± 1.4 (non–insulin-infused; $P < .03$ by ANOVA). Serum calcium was significantly decreased in amylin-treated animals (1.93 ± 0.04 *v* 2.30 ± 0.05 mmol/L, $P < .001$). The ability of insulin to promote R_d was decreased in the presence of amylin (12.0 ± 1.4 *v* 19.7 ± 2.7 mmol/kg/h per nmol/L insulin, $P < .03$), but the relationship between IR kinase activity and R_d was similar in the presence or absence of amylin (0.9 ± 0.1 *v* 1.1 ± 0.1 mmol/kg/h per fmol PO_4, P = NS). The data suggest that in vivo amylin may induce insulin resistance at or proximal to IR kinase.

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AMYLIN IS A RECENTLY described 37–amino acid peptide found in the pancreatic islets of man[1] and experimental animals[2] with non–insulin-dependent diabetes mellitus (NIDDM). Amylin levels in the plasma of normal subjects and patients with NIDDM have been reported to correlate with both basal and glucose-stimulated insulin levels.[3] Studies have demonstrated that infused exogenous amylin induces insulin resistance at both the hepatic and peripheral levels.[4,5] Studies in vitro have demonstrated that amylin impairs the ability of insulin to induce 2-deoxyglucose uptake into isolated muscle preparations.[6] Furthermore, amylin impairs glucose incorporation into glycogen[7] and promotes glycogenolysis.[8] These and similar findings have led to the hypothesis that amylin is contributory to the pathogenesis of NIDDM, although the significance of these findings is not established at physiologically relevant levels of amylin.[9,10] To study possible mechanisms whereby amylin might induce insulin resistance or otherwise alter glucose homeostasis in vivo, the hyperinsulinemic glucose clamp technique was used in the rat model in the presence of infused amylin. The effects of amylin thus studied in vivo were those on (1) overall glucose turnover, hepatic glucose output (HGO), and peripheral glucose uptake; (2) endogenous insulin production; (3) skeletal muscle insulin receptor (IR) kinase activity in vivo; and (4) serum calcium and insulin action.

MATERIALS AND METHODS

Materials

[^{125}I]Tyr^{A14}-insulin (porcine), D-[3-^{3}H]-glucose, and [γ-^{32}P]–adenosine triphosphate (ATP) (222 TBq/mmol) were purchased from New England Nuclear (Boston, MA). Porcine insulin, histone 2B type VIIS, and somatostatin (SRIF) were purchased from Sigma (St Louis, MO). Human amylin was obtained from Bachem (Torrance, CA). Salmon calcitonin was obtained from Rorer (Bramalea, Ontario, Canada). Wheatgerm agglutinin-agarose was obtained from Vector Laboratories (Burlingame, CA). Immobilized Protein A was purchased from Repligen (Cambridge, MA). All materials for sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis were from Bio-Rad (Richmond, CA). Human serum containing anti-IR antibody was kindly supplied by Dr Lawrence Mandarino (Pittsburgh, PA), and inactin was purchased from Byk-Gulden Pharmazeutika (Konstanz, Germany).

Experimental Animals

Male Sprague-Dawley rats weighing 200 to 250 g were obtained from the University of British Columbia Animal Care Center (Vancouver, BC). Studies were approved by the University Animal Care and Use Committee. Animals were housed in climate-controlled conditions with 12-hour alternating light-dark cycles and fed standard rat chow (49% carbohydrate, 5% fat, 23% protein, and 23% fiber) ad libitum, but were fasted overnight before study.

Clamp Studies in Rats

An adaptation of the glucose clamp technique described in humans[11] was used for rats, as described by Burnol et al.[12] After weighing, animals were anesthetized by single intraperitoneal

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injection of inactin 100 mg/kg body weight and placed on a warming pad. They underwent tracheostomy. The internal jugular vein and common carotid artery were then cannulated with fine-bore polyethylene tubing, which was periodically flushed with heparin (50 U/mL). During the first 15 minutes after surgery, baseline serum glucose measurements were obtained. Thereafter, the following were infused in various combinations (details of infusion protocols are described later): (1) insulin 14 pmol/kg/min from 0 to 120 minutes (preceded by a loading dose of 42, 28, and 21 pmol/kg/min for 1 minute each); (2) amylin 500 pmol/kg/min from 60 to 120 minutes; (3) SRIF 920 pmol/kg/min from 0 to 120 minutes; and (4) D-[3-^{3}H]-glucose 0.10 μCi/min from −15 to 120 minutes, after an initial 100× square-wave bolus over 1 minute, for isotopic determination of glucose turnover as described by Steele.[13] D-Glucose 20% was infused as needed to maintain serum glucose at mean values ranging from 6.6 to 7.8 mmol/L. Thirty microliters of arterial blood was sampled at 5-minute intervals for determination of serum glucose. At 60, 110, and 120 minutes, 200 μL blood was withdrawn for determination of steady-state serum insulin levels and tracer dilution. The rectus muscle was then exposed and minimally dissected only enough to allow rapid interposition of tongs precooled in liquid nitrogen. Frozen muscle was removed and immediately placed in liquid nitrogen, after which animals were killed by intravenous injection of pentobarbital 250 mg/kg.

Analytical Methods

Serum glucose was determined by a glucose oxidase technique on a YSI 23A glucose analyzer (YSI, Yellow Springs, OH). Serum insulin level was measured by double-antibody radioimmunoassay (ICN, Costa Mesa, CA) using porcine insulin standards. Serum calcium level was measured in a J-A Model 850 atomic absorption spectrophotometer (Jarrell-Ash, Waltham, MA). For determination of D-[3-^{3}H]-glucose concentrations, serum was diluted 1:4 with water and then added to an equal volume of perchloric acid at a final concentration of 2.5%. Proteins were precipitated by centrifugation at 2,000 × *g* for 10 minutes. Aliquots of supernatant were then dehydrated for 6 hours at 55°C and counted in a β-scintillation counter. Protein concentrations in wheatgerm eluates were measured by the Bradford dye method[14] using Bio-Rad reagent.

Isolation of IRs

IRs were purified as previously described.[15] In brief, the tissue underwent homogenization, solubilization, and ultracentrifugation in the presence of protease, phosphatase, and kinase inhibitors (5 trypsin inhibitory units/mL aprotinin, 5 mmol/L EDTA, 100 mmol/L NaF, 10 mmol/L tetrasodium pyrophosphate, 1.5 mg/mL bacitracin, 2 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L sodium orthovanadate (Na_3VO_4), and 1 mg/mL benzamidine). Fourfold-diluted ultracentrifuged supernatants were passed over wheatgerm affinity columns, washed, eluted in 0.3 mol/L *N*-acetylglucosamine in the presence of inhibitors,[15] and frozen at −70°C.

Binding Studies

Crude IR preparations were diluted with the same wheatgerm affinity column eluting buffer to a protein concentration of 0.25 mg/mL and added to an equal volume of a mixture containing ^{125}I-Tyr^{A14}-monoiodoinsulin (0.5 ng/mL) and increasing concentrations of unlabeled insulin in a total assay volume of 40 μL. After 12 to 15 hours at 4°C, IR complexes were precipitated according to the method described by Desbuquois and Aurbach[16] by addition of 22.5% polyethylene glycol and 0.45% γ-globulin initial concentrations. Counts in pellets in the presence of 5,000 ng/mL unlabeled insulin were subtracted as nonspecific binding. Immunodepletion binding studies in the presence of anti-IR antibody were performed as previously described.[15]

Kinase Assays

Individual IR preparations were adjusted to similar receptor number by relative binding potency, as described by Burant et al,[17] to contain approximately 90 fmol IR in 60 μL, and were added to either human serum containing anti-IR antibody (final serum dilution, 1:150) or normal human serum (1:50). After incubation at 4°C for 8 to 10 hours, samples were added to an equal volume of washed suspended Protein A and then immunoprecipitated.[18] Immunoprecipitated IRs were washed extensively to remove inhibitors, and then resuspended in 45 μL of a buffer containing 100 mmol/L NaCl, 2.5 mmol/L KCl, 1 mmol/L $CaCl_2$, 0.05% Triton X-100, 10% glycerol, 1 mmol/L Na_3VO_4, and 20 mmol/L HEPES, pH 7.4.

Following resuspension, 20 μL of a phosphorylation mixture was added to produce final concentrations of 1 mg/mL histone 2B, 5 mmol/L $MnCl_2$, 10 mmol/L $MgCl_2$, 0.5 mmol/L cytidine triphosphate, 2 mmol/L Na_3VO_4, and 4 μmol/L ATP (78 Ci/mmol). The reaction was allowed to proceed for 5 minutes at 4°C and was then stopped with an equal volume of a buffer containing 100 mmol/L ATP, 100 mmol/L dithiothreitol, 10 mmol/L EDTA, 1% SDS, 10% glycerol, 0.05% bromophenol blue, and 0.5 mol/L Tris base, pH 6.8, in final dilution, followed by boiling for 3 minutes. Supernatants were loaded onto 14% gels and submitted to SDS–polyacrylamide gel electrophoresis according to the method reported by Laemmli,[19] followed by incubation in 0.85 mol/L KOH at 55°C for 2 hours to remove non–phosphotyrosine–containing phosphoproteins, as described by Cooper and Hunter.[20] To account for variable alkali lability, IRs from all experimental groups were included in each gel. They were then dehydrated in a solution of 40% ethanol, 1% glycerol, and 10% acetic acid for 2 hours before drying. After autoradiography, bands were excised. Counts in bands were determined by subtraction of counts in an equal area of the same lane in which no discrete phosphoproteins were identified. Specific IR-mediated counts incorporated into histone 2B were calculated by subtraction of counts obtained in the presence of normal human serum from those obtained in the presence of antibody.

Statistical Methods

A two-sample *t* test was used for continuous variables. Data were analyzed by ANOVA using the Quick CSS program (Statsoft, Tulsa, OK).

RESULTS

Glucose Clamp Studies

Results of hyperinsulinemic clamps in the presence and absence of amylin from 60 to 120 minutes are listed in Table 1. In the presence of amylin versus saline, overall glucose disposal (R_d) was significantly reduced (mean ± SE: 5.0 ± 0.2 *v* 8.5 ± 0.6 mmol/kg/h, $P < .001$). HGO was no longer fully suppressed by insulin in amylin-treated groups. A negative value was obtained for HGO in the saline-treated group, because cold glucose infusate was not spiked with 3-^{3}H-glucose in these studies. When SRIF was infused simultaneously with amylin, serum insulin levels were significantly reduced (408 ± 15 pmol/L) in comparison to levels in animals infused with saline (636 ± 50, $P < .01$) or amylin alone (661 ± 89, $P < .05$). However, the effect of

Table 1. Results of Hyperinsulinemic Glucose Clamp Studies

	Amylin	Saline	Amylin + SRIF
No.	6	5	5
Weight (g)	224 ± 3	234 ± 8	245 ± 2
Steady-state glucose (mmol/L)	7.2 ± 0.4	6.6 ± 0.1	7.1 ± 0.3
Steady-state serum insulin (pmol/L)	661 ± 89*	636 ± 50†	408 ± 15
R_d (mmol/kg/h)	5.0 ± 0.2‡	8.5 ± 0.6	4.2 ± 0.4‡
HGO (mmol/kg/h)	1.4 ± 0.2‡	−1.9 ± 0.3	1.4 ± 0.5‡

NOTE. Values are the mean ± SE.
*$P < .05$, †$P < .01$: *v* SRIF-treated group.
‡$P < .001$ *v* saline group.

amylin on R_d or HGO persisted in the presence of SRIF, as shown in Table 1.

Binding Studies

IR binding data are listed in Table 2. Receptor recovery in wheatgerm eluates ranged from 1.4 to 1.8 pmol/mg eluate protein, and was similar for all groups of animals. Similarly, IR insulin binding affinity was not significantly different among any of the treatment groups, with the ED_{50} of unlabeled insulin for displacement of ^{125}I-insulin ranging from 1.69 to 2.14 nmol/L. Although IR number and insulin binding affinity were similar among groups, it is possible that pretreatment with amylin altered affinity of the IR for the anti-IR antibody used to immunoprecipitate the IR for subsequent kinase assay. Thus, differences in the number of IRs precipitated might have accounted for differences in kinase data, despite the use of the same number of receptors in IR preparations. To exclude this possibility, immunodepletion studies were performed. Insulin binding activity of IR preparations obtained from both amylin-treated and saline-treated animals was measured before and after incubation with the anti-IR antibody. As shown in Fig 1, there was no difference in the percent depletion of IR from IR preparations by anti-IR antibody in the two groups.

Kinase Studies

The effect of amylin infusion on IR kinase activity in vivo is illustrated in Figs 2 and 3. Muscle IR kinase activity was decreased in amylin-treated animals. This was true whether IR kinase activity was only adjusted for IR number in the in vitro assay or further quantified per 1 nmol/L serum insulin under clamp conditions (Fig 2). Furthermore, when amylin was infused in the absence of exogenous insulin infusion, similar findings were obtained (Fig 3). In analyzing independently the effect of the association between the presence of

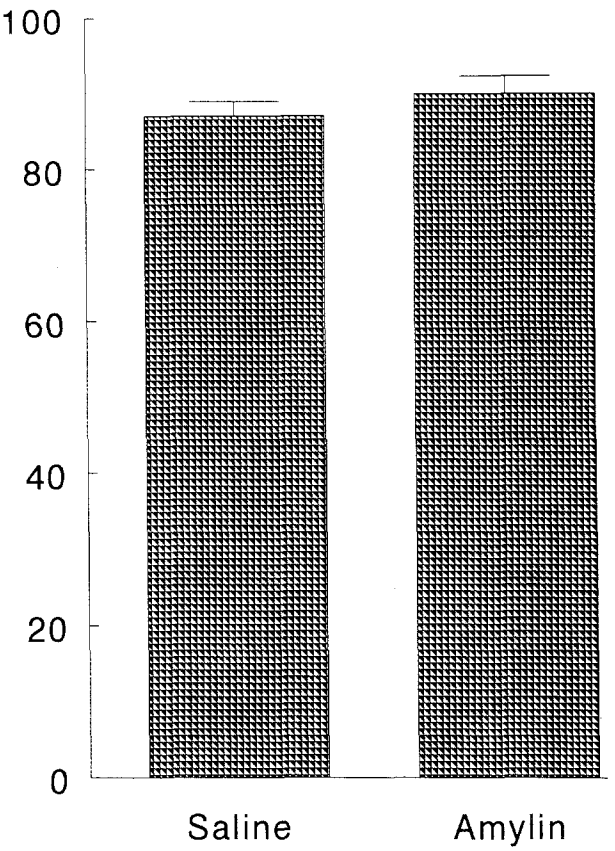

Fig 1. Effect of immunodepletion by anti-IR antibody on insulin-binding activity (mean ± SE) of IR preparations from amylin-treated and saline-treated animals. Percent total insulin binding activity specifically precipitated after incubation with anti-IR antibody was 87.1 ± 1.9 (saline-treated) and 89.9 ± 2.4 (amylin-treated). *P* = NS.

amylin and the alteration in IR kinase activity by ANOVA, this was significant at the level of P less than .03. In non–insulin-infused animals, it was necessary to clamp steady-state serum glucose at the level of those animals receiving both amylin and SRIF, since their glucose levels were slightly but consistently higher. As can be seen from the data in Table 3, this finding is attributable to two factors: first, the inhibition of endogenous insulin production by SRIF (and the lack of such an inhibition by amylin alone), and second, the induction of insulin resistance by amylin, which results in significantly greater HGO in the basal state. In the presence of SRIF, IR kinase activity was slightly decreased both with and without infused insulin; however, this presumably reflects the decreased serum

Table 2. Insulin Binding Characteristics of Receptors Isolated From Rectus Muscle

	Insulin + Saline	Insulin + Amylin	Saline Only	Amylin Only
No.	5	11	6	12
Receptor no. by Scatchard analysis (pmol/mg protein)*	1.5 ± 0.4	1.8 ± 0.3	1.4 ± 0.5	1.7 ± 0.2
ED_{50} of insulin to displace ^{125}I-insulin (nmol/L)	2.14 ± 0.55	2.08 ± 0.23	1.69 ± 0.23	1.84 ± 0.19

NOTE. Values are the mean ± SE.
*x-intercepts for individual Scatchard plots.

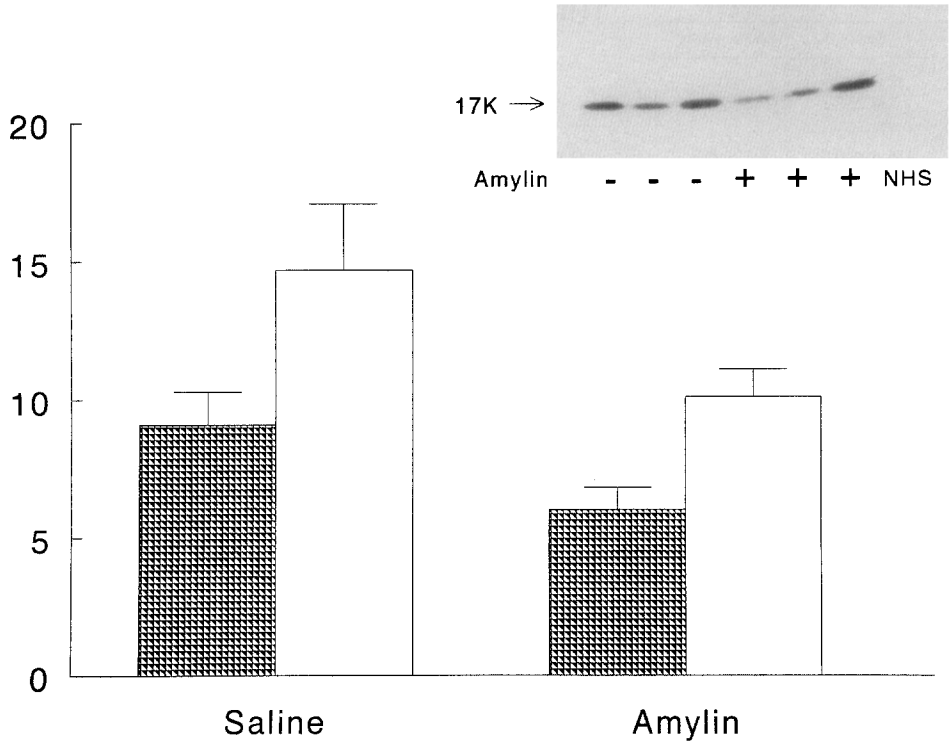

Fig 2. Effect of amylin on skeletal muscle IR kinase activity (mean ± SE) activated by insulin in vivo. (▨) fmol phosphate into histone per 5 minutes (saline, 9.1 ± 1.2; amylin, 6.0 ± 0.8); (□) fmol phosphate into histone per nmol/L steady-state serum insulin during glucose clamps (saline, 14.7 ± 2.4; amylin, 10.1 ± 1.0). Inset: representative histone bands from this experiment. First three bands derived from saline-infused animals; right three bands derived from amylin-infused animals. NHS, normal human serum. Fig 3 for statistical interpretation.

insulin levels, since IR kinase activity per 1 nmol/L serum insulin was unchanged (data not shown).

The data were further analyzed as shown in Fig 4. R_d per 1 nmol/L serum insulin was reduced by 39% in the presence of amylin (12.0 ± 1.4 *v* 19.7 ± 2.7 mmol/kg/h per nmol/L insulin, $P < .03$). However, R_d per femtomole PO_4 incorporated into histone was unchanged (0.9 ± 0.1 in the presence of amylin *v* 1.1 ± 0.1 mmol/kg/h per fmol PO_4 in the absence of amylin, P = NS). This suggests that steps in insulin signal transduction proximal to or at the level of the IR kinase were impaired by amylin, whereas more distal steps were not.

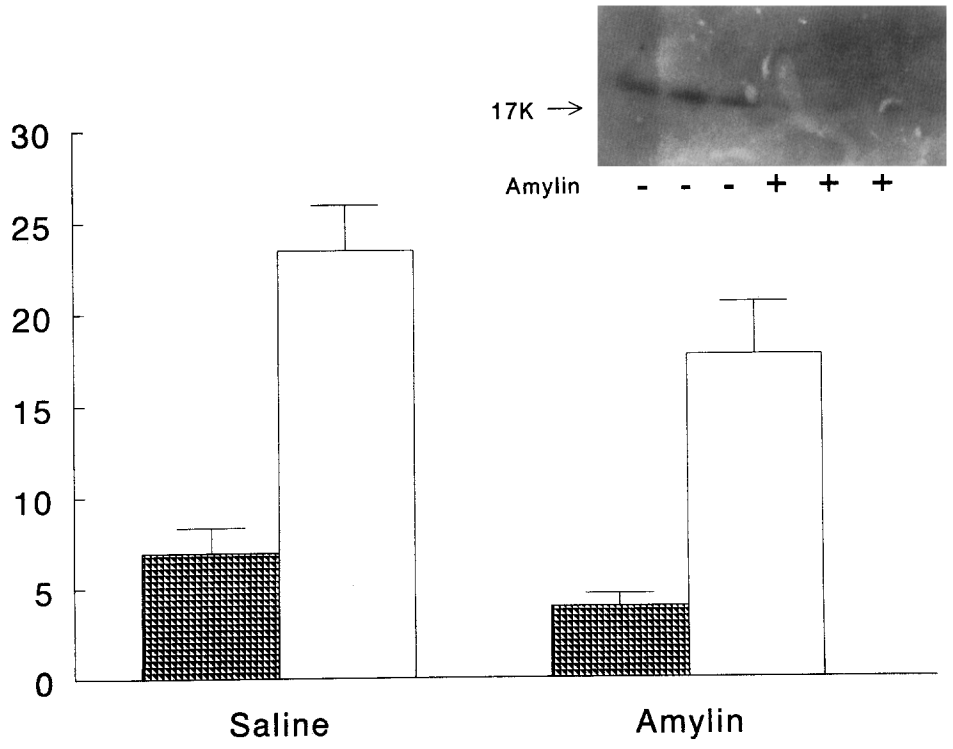

Fig 3. Effect of amylin on skeletal muscle IR kinase activity (mean ± SE) in the absence of exogenously infused insulin. Glucose increased slightly but consistently when amylin was infused, and thus glucose was also clamped at this level in animals receiving saline. Steady-state conditions attained as in Table 3. Data otherwise obtained as described in Fig 2 (including inset) and similarly labeled. Results left to right: 6.9 ± 1.4, 23.5 ± 2.5, 3.9 ± 0.7, and 17.7 ± 2.9. When data comprising Figs 2 and 3 were analyzed by ANOVA for an effect of amylin on IR kinase activity, $P < .03$.

Table 3. Results of Basal Glucose Clamp Studies

	Amylin	Saline	Amylin + SRIF
No.	7	6	5
Weight (g)	243 ± 5	245 ± 7	232 ± 5
Steady-state glucose (mmol/L)	7.3 ± 0.3	7.3 ± 0.4	7.8 ± 0.2
Steady-state serum insulin (pmol/L)	260 ± 30*	310 ± 57†	151 ± 11
R_d (mmol/kg/h)	3.8 ± 0.4	7.2 ± 1.1‡	3.6 ± 1.0
HGO (mmol/kg/h)	0.7 ± 0.4§	−1.3 ± 0.7	2.2 ± 0.6‖

NOTE. Values are the mean ± SE.
*P < .02, †P < .05: v SRIF-treated group.
‡P < .02 v amylin-treated group.
§P < .05, ‖P < .01: v saline-treated group.

To clarify further this relationship, linear regression of total R_d against IR kinase activity was performed for amylin-treated and saline-treated rats. The data are shown in Fig 5 and confirm that although absolute IR kinase activity was lower in amylin-treated rats, for a given IR kinase activity, R_d was similar in both amylin-treated and saline-treated animals, with the slope of the regression line being 0.51 for saline-treated and 0.38 for amylin-treated animals. Correlation coefficients were also virtually the same (r = .72, P = .028 for amylin-treated, and r = .71, P = .031 for saline-treated).

Serum Calcium Studies

In view of the known effects of amylin on calcium metabolism,[21] the effect of amylin infusion on serum calcium was studied. The data are shown in Fig 6. In amylin-treated animals, serum calcium after 60 minutes was significantly lower than in saline-treated animals (1.93 ± 0.04 v 2.30 ± 0.05 mmol/L, P < .001). To determine whether the calcium-lowering effect of amylin played a role in the induction of insulin resistance, similar studies were undertaken with infusion of salmon calcitonin in

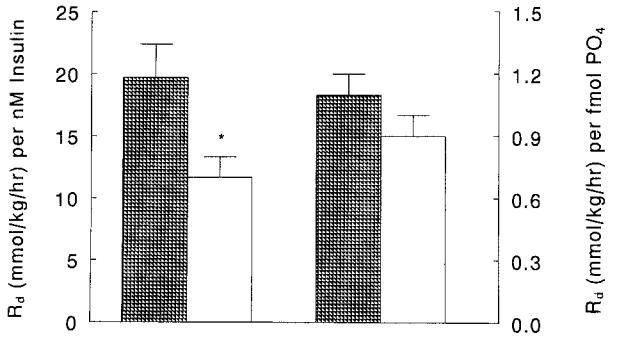

Fig 4. Effect of amylin on R_d in vivo per unit serum insulin during euglycemic clamps and on R_d per unit in vivo activation of muscle IR kinase (mean ± SE). Data from both insulin-infused and non–insulin-infused rats were included for analysis. Left pair of bars: R_d in clamps expressed per mmol/kg/h per nmol/L insulin in serum at steady state. Values are 19.7 ± 2.7 (▨, without amylin, n = 9) and 12.0 ± 1.4 (□, with amylin, n = 9); *P < .03. Right pair of bars: R_d in clamps expressed per IR-mediated fmol of phosphate incorporated into histone during the kinase assay. Values are 1.1 ± 0.1 (▨, without amylin) and 0.9 ± 0.1 mmol/kg/h/fmol (□, with amylin); P = NS.

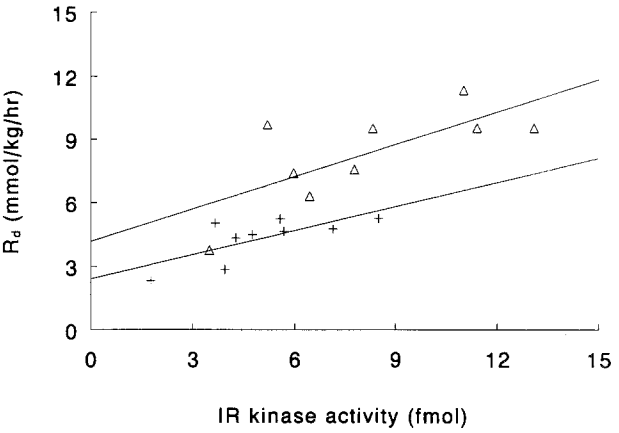

Fig 5. Relationship between total R_d and muscle IR kinase activity in saline-treated (△) and amylin-treated (+) animals. Saline-treated: slope = 0.51, r = .71, P = .031; amylin-treated: slope = 0.38, r = .72, P = .028.

concentrations that produced a significant reduction in serum calcium level (1.88 ± 0.07 mmol/L, n = 5). However, calcitonin did not induce insulin resistance (R_d = 7.6 ± 0.2 v 8.5 ± 0.6 and HGO = −2.7 ± 0.2 v −1.9 ± 0.3 mmol/kg/h for calcitonin and saline, respectively, P = NS).

DISCUSSION

Induction of insulin resistance by amylin under euglycemic glucose clamp conditions was initially reported by Molina et al[4] and Sowa et al.[5] Our findings are similar, namely that a significant reduction in insulin sensitivity occurs in both the suppression of HGO and peripheral R_d. Furthermore, we have been able to demonstrate that amylin induces both of these effects at basal postabsorptive insulin levels in the absence of exogenous insulin infusion. Concomitant infusion of SRIF demonstrates that this effect is not the result of a reduction in pancreatic insulin production by amylin, since serum insulin levels do not

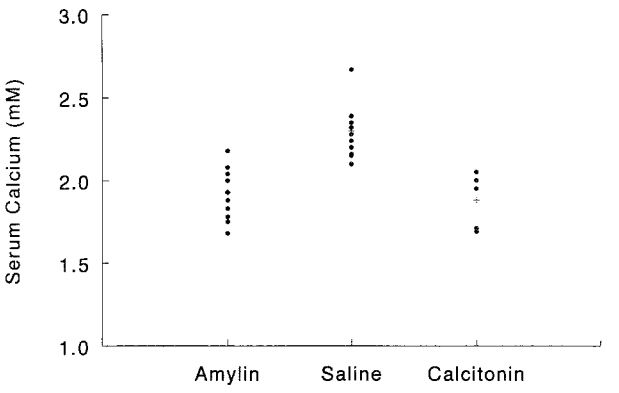

Fig 6. Effect of amylin on serum calcium concentration (mean ± SE) in the rat during glucose clamp studies. Serum was obtained after 60 minutes of infusion of amylin or saline. Calcium levels were 2.30 ± 0.05 and 1.93 ± 0.04 mmol/L for saline and amylin, respectively, P < .001. When serum calcium was decreased similarly by infusion of salmon calcitonin, R_d was not significantly altered (8.5 ± 0.6 v 7.6 ± 0.2 mmol/kg/h for saline and calcitonin, respectively, P = NS).

decrease in the presence of amylin alone, whereas the simultaneous infusion of SRIF demonstrates a substantial suppressible component in both the insulin-infused and basal studies.

Amylin has been reported to suppress endogenous insulin production in isolated rat islets (but not in the perfused rat pancreas) only at high concentrations,[22] whereas several investigators have reported no effect of amylin on basal or glucose-stimulated insulin secretion either in cultured cell systems[23,24] or in vivo.[24,25] The present study further confirms a significant level of SRIF-suppressible endogenous insulin production under euglycemic clamp conditions, which was unaffected by amylin.

Previous investigators have reported no effect of amylin on IR kinase activity.[26] However, these studies used a different approach from that used here, in that amylin was incubated with isolated IR in the presence of insulin in vitro. Thus, any effect exerted by amylin on IR kinase activity would be mediated via a direct action of amylin on the IR itself, either by directly binding to the insulin-binding site or allosterically affecting the receptor at some other site. Given the weak structural homology of amylin with insulin[1] and the fact that its close relatives calcitonin gene-related peptide and calcitonin act through a different class of receptor from the IR,[27,28] it is not surprising that amylin fails to exert a direct effect on IR kinase activity in vitro. The present study involves the exposure of the IR to insulin and amylin in vivo, where a number of regulatory mechanisms may be brought to bear on the IR in the intact physiologic environment.[15,18] Subsequently, the in vitro assay of IR kinase activity occurs without further exposure of the IR to insulin or amylin in vitro, and the activation state of the IR in vivo is extrapolated from its kinase activity toward a phosphoacceptor substrate in vitro. For example, using this technique, we recently reported that acute increases in serum glucose level altered IR kinase activity in vivo, whereas exposure of isolated IRs to altered glucose concentrations in vitro produced no such effect.[29] When studied in this way, in vivo–activated skeletal muscle IR kinase activity is significantly reduced when amylin is co-infused with insulin. Amylin levels achieved in this study will be greater than those likely to occur physiologically in insulin-resistant states. Infusion of amylin at low rates (25 pmol/min) has been shown to induce hepatic insulin resistance in rats[26] but not in dogs.[30] Furthermore, no effect of amylin was demonstrable on glucose output of isolated[31] or in situ–perfused[32] rat liver. Young et al[33] reported that amylin elicited a marked increase in plasma lactate in fasted rats, which was not seen with glucagon administration, suggesting that amylin may increase HGO by increased provision of gluconeogenic precursors to the liver. Knowledge of the effect of physiologic concentrations of amylin on both liver and muscle IR kinase activity in this system will thus be of further interest.

Our data suggest that amylin's ability to induce insulin resistance in skeletal muscle is mediated by a defect in the IR kinase or at a step in insulin signaling proximal to it. As shown in Fig 4, there is an approximately 40% reduction in the activation of the IR kinase per 1 nmol/L insulin in serum during euglycemic clamps in the presence of amylin. However, when R_d is determined per unit of IR kinase activity (ie, per femtomole of PO_4 incorporated into histone), this is similar in both amylin-treated and control animals, whereas total R_d per unit IR kinase activity correlated equally well in both amylin- and saline-treated animals. Frontoni et al[34] have reported that amylin infusion in rats during euglycemic clamps results in a reduction of glycogen synthesis, an accumulation of glucose-6-phosphate, and increased glycolysis. The results of our study suggest that such postreceptor effects may have occurred secondary to abnormalities in kinase activation or a step proximal to it.

The hypocalcemic effect of amylin seen in these studies has also been described in dogs during euglycemic clamps by Kassir et al[30] and in conscious rats by Furnsinn et al.[35] In both studies, hypocalcemia was elicited in the absence of insulin resistance. Taken with our own, these results confirm that the hypocalcemic effect of amylin per se does not mediate insulin resistance. In addition, amylin has also been shown to possess vasodilator and hypotensive actions.[36,37] We did not measure blood pressure in these studies; however, reduction of blood pressure within the physiologic range has not consistently been reported to result in altered insulin action.

The mechanism whereby amylin alters IR kinase activity in vivo is unclear. Two possible mechanisms addressed in our study, namely reduction in serum calcium or endogenous insulin production, do not appear to provide the answer. Other possibilities include a direct or indirect inhibitory effect on the IR kinase by the receptor for amylin, or by an amylin-induced increase in tyrosine phosphoprotein phosphatase activity or altered internalization and processing of activated IR. Further studies are required to address this issue.

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