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Amylin injection causes elevated plasma lactate and glucose in the rat

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Intravenous injections of 25.5 nmol rat amylin into fasted anesthetized rats caused a rapid increase in plasma lactate followed by an increase in plasma glucose; there was a transient fall in blood pressure. Subcutaneous injection of 25.5 nmol amylin also caused increases in lactate and glucose but did not change blood pressure. Similar responses were observed during somatostatin infusion and in the absence of changes in catecholamines. These results fit with a scheme in which amylin elicits muscle glycogenolysis, release of lactate, and increased hepatic gluconeogenesis due to increased supply of substrate.

Gluconeogenesis; Rat skeletal muscle; Glycogen; Glucose; Lactate; Blood pressure

1. INTRODUCTION

Amylin is a 37 amino acid protein which is the major constituent of the islet amyloid found in patients with type 2 (non-insulin-dependent) diabetes mellitus [1]. Amylin is synthesized in islet β-cells and secreted along with insulin in response to nutrient stimuli [2]. Amylin has reported biological effects in a range of tissue targets including skeletal muscle [3], bone [4], liver [5] and the pancreas [6]. In skeletal muscle, amylin was found to inhibit the incorporation of radiolabelled glucose into glycogen [7], to inhibit glycogen synthase [8] and to activate glycogen phosphorylase [9]. During euglycemic hyperinsulinemic glucose clamp studies, amylin reduces peripheral glucose disposal and opposes insulin-mediated suppression of hepatic glucose production [10,11].

Amylin has also been reported to have an hypotensive action [12,13]. Previous studies of amylin actions in vivo have mostly looked at the ability of amylin to modulate insulin-mediated metabolic changes, and none of the studies have included measurement of both metabolic and cardiovascular variables in the same preparation. While other studies have generally looked at the ability of amylin to modulate insulin-mediated metabolic changes, the present study reports the metabolic response to amylin alone, with or without somatostatin infusions to inhibit secretion of other pancreatic and pituitary hormones. We have used lightly halothane- anesthetized rats fasted for 18 h. These animals are depleted in liver glycogen due to the fasting state, and provide a stable preparation for measurements over many hours.

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Both intravenous and subcutaneous injections of 25.5 nmol amylin cause rapid increases in plasma lactate, followed by increases in plasma glucose. We have proposed that amylin acts primarily to cause glycogenolysis in skeletal muscle, with increased production of glucose-6-phosphate, increased glycolysis, and increased production and release of lactate; this lactate could then serve as a substrate for hepatic gluconeogenesis. As one test of this idea, lactate infusions were given to mimic the increases in plasma lactate caused by amylin, and the glucose increment thus produced was compared with that evoked by amylin. The data reported here are consistent with this proposal. The metabolic actions of amylin appear to be independent of changes in blood pressure, insulin, or the main, rapidly acting counter-regulatory hormones.

2. MATERIALS AND METHODS

2.1. *Animals*

Male Harlan–Sprague–Dawley rats (body mass 338±7 g, age 90±3 days) were housed at 22.7±0.8°C in a 12:12 hour light:dark cycle (experiments being performed during the light cycle) and fed and watered ad libitum (Diet LM-485, Teklad, Madison, WI). Experiments were performed after a fast of 19±0.4 h. There were 5 treatment groups. All animals were treated identically in regard to surgery, instrumentation, blood withdrawal and infusion of fluids. The treatment groups were as follows:

1. Intravenous amylin bolus with somatostatin infusion (n=6). After 2-hours infusion with somatostatin (S-9129, Sigma, St Louis, MO) at 3.4 nmol/h, which then continued throughout the experiment, animals were injected with a 100 μl 150 mM NaCl containing 25.5 nmol freshly dissolved rat amylin (lot# ZG485, Bachem, Torrance, CA). The biological activity of peptide to be used in this study was first verified using the soleus muscle-based assay [14] (EC_{50}=6.7±1.5 nM).
2. Intravenous amylin bolus without somatostatin infusion (n=7). Animals were injected with an intravenous bolus of 25.5 nmol rat amylin in 100 μl saline as for group 1.

3. Subcutaneous amylin injection without somatostatin infusion (n=5). Animals were injected under the abdominal skin with 25.5 nmol rat amylin in 100 μl saline.
4. Intravenous primed/continuous infusion of sodium lactate (n=3). Animals were infused intravenously with 875 mM Na lactate in saline (pH 7.0) at 1.5 ml/h for 1 h. A 0.5 ml bolus was delivered 30 min after the start of infusion. This protocol attempted to replicate the plasma lactate profile observed following amylin administration.
5. Controls (n=12). Rats pre-infused with somatostatin (as in group 1) were injected with 25.5 nmol amylin that had been autoclaved at 121°C for 90 min (n=3) or with saline alone (n=3). Rats not infused with somatostatin were injected with saline alone (n=6). Since there were no differences between responses to any of these treatments, data have been pooled into a single control group.

2.2. *Surgery/instrumentation*

Anesthesia was induced with 5% halothane, maintained at 2% during surgery and at 0.8–1% during metabolic recordings. Tracheotomy and cannulation of the right femoral artery and saphenous vein enabled recording of arterial pressure (Spectramed P23XL transducer, model 13-4615-58 amplifier, Gould, Cleveland, OH) and intravenous infusion. Signals for mean arterial pressure were sampled and stored with 12-bit precision at 1 Hz using a computerized data acquisition system (DT2801A A/D converters, DataTranslation, Marlboro, MA; AST Premium 386 computer, AST Research, Irvine, CA; Labtech Notebook software, Laboratory Technologies Corp, Wilmington, MA). Colonic temperature was measured and controlled using a thermistor probe and controller (Model 73A, YSI, Yellow Springs, OH) connected to a heated operating table.

2.3. *Chemical analyses*

Arterial samples were drawn 0.5, 0.25 and 0 h before bolus injection, and 0.5, 1, 1.5, 2, 3 and 4 h after injection. Samples were collected into heparinized capillaries, centrifuged, and the separated plasma analyzed for glucose, lactate, insulin and in some cases, catecholamines and amylin. All chemicals were of analytical grade or better.

Glucose and lactate were analyzed immediately by immobilized enzyme chemistries (glucose oxidase, L-lactate oxidase, Analyzer model 2300-STAT, YSI, Yellow Springs, OH).

Insulin was determined by radioimmunoassay (Micromedic human insulin RIA kit, ICN Biomedicals, Horsham, PA), sensitivity 6 pM, cross-reactivity to rat insulin 89.5%. Rat amylin was determined by radioimmunoassay of whole plasma (antibody RAS 7323-N, lot# 019166-1, Peninsula Laboratories, Belmont, CA) using as standards, rat amylin (lot #ZG485, Bachem, Torrance, CA) added to plasma of rats previously made diabetic by intravenous injection of 65 mg/kg streptozotocin.

Plasma catecholamines (epinephrine and norepinephrine) were measured at 0, 2 and 4 h post-injection using HPLC with electrochemical detection following plasma extraction with alumina. A modification of the method of Weicker et al. [15], whereby internal standard (dehydroxybutyric acid) was added to plasma prior to extraction enabled analysis of 50 μl samples with an intra-assay coefficient of variation of 8.1%.

2.4. *Numerical methods*

Results are presented as means ±SEM. Pairwise comparisons were performed using paired or unpaired t-test routines contained within the SYSTAT program [16].

3. RESULTS AND DISCUSSION

3.1. *Intravenous injection of amylin*

Fig. 1 shows the plasma lactate and glucose concentrations that follow intravenous injections of 25.5 nmol rat amylin (open circles). Amylin elicited an approximately 3-fold rise in lactate, which was maximal by 30 min, and returned to the basal level at 3 h. There was also a rise in glucose from 5.9±0.3 mM to 11.0±0.6 mM. It was maximal between 1 and 2 h and was still above the control level at 4 h. These data, and more detailed measurement of the early time course (Wang and Young, unpublished data) indicated that the plasma lactate increase precedes that of glucose. The dotted lines show the control response in animals injected with saline vehicle alone; there was a slow rise in plasma glucose, consistently seen during the 6 h following surgery, but no change in plasma lactate.

Amylin activates muscle glycogen phosphorylase by conversion to the active a-form [8,9]. This action of amylin may contribute to the increased muscle lactate production in vitro [7], increased glucose-6-phosphate concentration [17], and decreased total glycogen content [17] following amylin stimulation. The appearance of lactate in the plasma following amylin administration in this study is consistent with amylin-induced muscle glycogenolysis.

3.2. *Intravenous amylin injections during infusion of somatostatin*

The increases in lactate and glucose concentration elicited by amylin could have been accompanied by, or in part caused by, changes in other hormones, such as insulin and glucagon. Therefore, the effects of intravenous amylin were examined in animals infused with

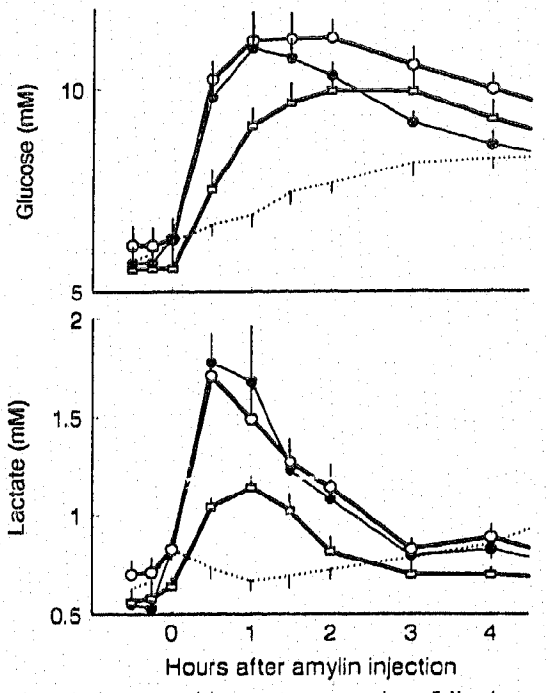

Fig. 1. Plasma glucose and lactate concentrations following amylin administration. Arterial glucose (upper panel), and lactate concentration (lower panel) following 25.5 nmol amylin: i.v. with somatostatin preinfusion (●); i.v. without somatostatin (○); subcutaneous (□). The broken line shows the control response. Symbols represent means ±SEM. n=5–12.

Table I

Hormonal levels

	Time (h)	Insulin (pM)	Epinephrine (nM)	Norepinephrine (nM)
Amylin injected (somatostatin preinfusion)				
	0.0	111.0 ± 44.4	2.67 ± 0.55	3.99 ± 0.29
	2.0	152.4 ± 40.8	2.73 ± 1.09	2.38 ± 0.97
	4.0	99.6 ± 27.0	1.91 ± 0.82	2.38 ± 1.12
Control				
	0.0	133.2 ± 43.8	3.06 ± 0.71	3.79 ± 0.63
	2.0	170.4 ± 25.8	4.15 ± 0.38	3.79 ± 0.34
	4.0	218.4 ± 49.8	4.09 ± 0.98	3.99 ± 0.53

somatostatin, which is established as an inhibitor of endocrine secretion, including secretion of glucagon and insulin from pancreatic islets. The results are shown in Fig. 1, by the closed circles. 'Pancreatic clamping' by somatostatin changed the response very little from that observed in the absence of somatostatin; the return of glucose concentrations towards control levels was somewhat faster. Table I shows that insulin levels did not increase following amylin injection in somatostatin-infused animals. In contrast, we found in a subsequent study (using a different insulin assay) that plasma insulin was increased 2.3-fold 1 h after intravenous injection of amylin. Thus, it appears that somatostatin infusion inhibited insulin secretion in these experiments. Owing to necessary limitation of sample volumes, glucagon levels were not measured. However, glucagon does not increase plasma lactate and so could not have produced that particular amylin effect under the present experimental conditions.

Also shown in Table I are measurements of plasma catecholamines. In the somatostatin-infused animals, amylin injections did not lead to an increase in epinephrine or norepinephrine; the levels remained close to control values. Thus, it appears that amylin's actions do not result from increased circulating catecholamines. This view is supported by the failure of amylin infusion to change such hormones [11].

3.3. *Blood pressure response to intravenous amylin injection*

Changes in mean arterial pressure following amylin administration are shown in Fig. 2. Rats receiving an intravenous bolus of 25.5 nmol amylin (with or without prior somatostatin infusion) showed a fall in mean arterial pressure of around 35 mmHg within 1 min of injection. Blood pressure then returned to preinjection levels over the next 30 min.

The hypotensive action of amylin in the present studies is consistent with an acute action at the vascular CGRP (CGRP$_1$) receptor. Amylin is nearly 50% homologous with calcitonin gene-related peptide, the most potent vasodilator yet described [18]. Indeed, we [12] and others [13] have demonstrated that the hypotensive effect of amylin can be blocked by the co-administration of the CGRP-antagonist, $^{8\text{-}37}$hCGRP.

3.4. *Plasma amylin concentrations and sequence of amylin-mediated events*

The time course of plasma amylin concentration following intravenous injection of 25.5 nmol was assessed in 2 rats which were treated identically to those in group 2, except that plasma samples were drawn more frequently. The decay curve could be fitted to 2 exponentials, the principal one decaying from an initial extrapolated plasma concentration of 33 nM with a $t_{1/2}$ of 12.5 min, the minor second one decaying with a $t_{1/2}$ of 69 min. The rapidity and transience of the hypotension and appearance of lactate after amylin injection mirrors the decay of plasma immunoreactivity. The slower and more prolonged increases in plasma glucose following amylin injection and following lactate infusion may reflect a limited capacity to process the lactate and glucose released into the blood. The observed sequence of changes in plasma lactate and glucose indicate that the hyperglycaemia may be a consequence of the hyperlactaemia, but that the reverse is unlikely to be the case.

3.5. *Subcutaneous infection of amylin*

Fig. 1 shows (open squares) the increases in plasma lactate and glucose following subcutaneous injection of 25.5 nmol amylin. There was an approximately 2.2-fold increase in plasma lactate which preceded an increase of plasma glucose from 5.57±0.44 mM to 9.96±0.51 mM. These responses were slightly delayed compared with response to intravenous injections – as expected

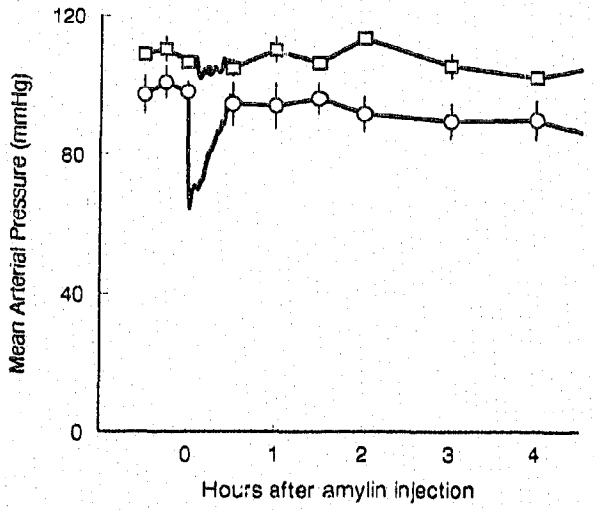

Fig. 2. Acute blood pressure response. Mean arterial pressure for rats injected with 25.5 nmol amylin: (○, n=6) intravenous injection (no somatostatin preinfusion), (□, n=2) subcutaneous injection. Symbols are means ±SEM. The mean continuous record is plotted for the first 30 min in each group.

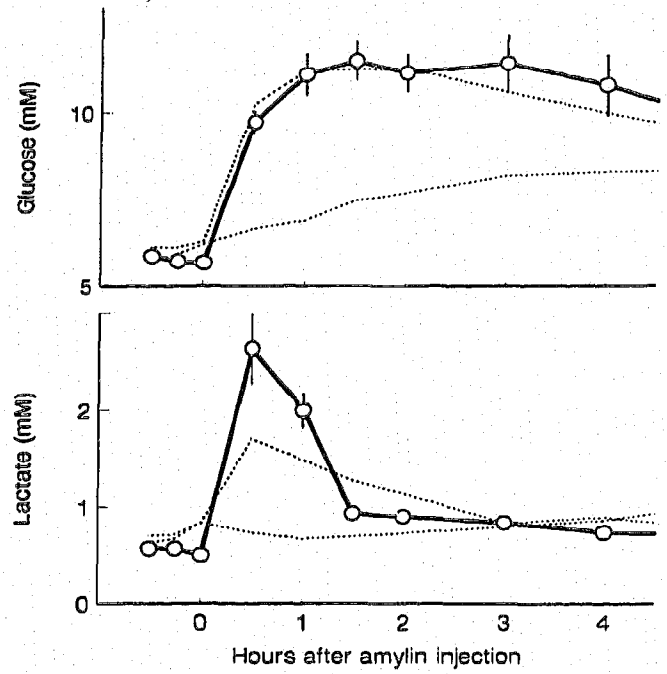

Fig. 3. Plasma glucose and lactate concentrations following lactate infusion into fasted rats. Increase in plasma glucose (upper panel) and lactate (lower panel) following infusion of a total of 1.75 mmol Na-lactate into fasted rats. The broken lines define the response to amylin and saline as shown in Fig. 1. Symbols are means ±SEM. n=3.

from the delayed access of amylin to the circulation. Simultaneous measurement of blood pressure showed no change, as shown in Fig. 2. Thus, amylin can evoke substantial increases in plasma lactate and glucose without any hypotensive effect. This is relevant since blood pressure reduction per se can contribute to increases in plasma lactate and glucose, as we saw when the amylin-evoked transient hypotension was mimicked using a train of pulse injections of phentolamine, an α-adrenergic blocker (data not shown).

3.6. *Response to sodium lactate infusion*

To see whether the amylin-stimulated rise in blood glucose could have been caused mainly by the increased lactate, rats were given a primed/continuous infusion of Na-lactate to produce a rise in plasma concentration comparable to that produced by amylin injection. This lactate infusion resulted in an increase in plasma glucose similar to that observed following amylin injection, as shown in Fig. 3. There was no change in mean arterial pressure. Thus, it appears that the amylin-evoked increase in lactate, representing increased availability of gluconeogenic substrate, could be a major cause of the increase in blood glucose; intraportal lactate infusions into fasted rats increase both plasma glucose concentration and liver glycogen [19] and lactate is taken up by perfused livers from fasted rats in direct proportion to its plasma concentration [20]. Whether amylin acts directly on the liver is currently unclear. While amylin was reported not to directly stimulate hepatic glycogenolysis [21,22], it nonetheless appears to inhibit insulin's hepatic effects, including suppression of glucose production [10,11] and stimulation of hepatic glycogen synthase [5].

3.7. *Conclusion*

Amylin, independent of the effects of counter-regulatory hormones or of changes in blood pressure, causes increases in plasma lactate concentration and slower increases in plasma glucose concentration. This sequence of changes, combined with the increases in plasma glucose seen following lactate infusion, suggests that the observed increases in plasma glucose are a consequence of amylin-induced hyperlactaemia.

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