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Selective amylin antagonist suppresses rise in plasma lactate after intravenous glucose in the rat

Evidence for a metabolic role of endogenous amylin

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Abstract

Data presented here provide the first demonstration that circulating amylin regulates metabolism in vivo, and support an endocrine hormonal role that is distinct from its autocrine action at pancreatic islets. When rats were pre-treated with the potent amylin antagonist AC187 ($n = 18$), and then administered a 2 mmol glucose load, the rise in plasma lactate was less than in rats administered glucose only ($n = 27$; $P < 0.02$). When rats were treated so that plasma glucose and insulin profiles were similar ($n = 8$), the increase in plasma lactate in the presence of AC187 was only 50.3% as high as the increase when AC187 was absent ($P < 0.001$). These experimental results fit with the view that some of the lactate appearing in plasma after a glucose load comes from insulin-sensitive tissues. The experiments also support the view that an important fraction of the increase in lactate depends on processes inhibited by a selective amylin antagonist, most likely amylin action in muscle.

Key words: Insulin; Post-prandial; Glycogenolysis; Glycolysis; AC187

1. Introduction

Amylin is a 37-amino acid peptide [1] co-secreted with insulin from pancreatic β-cells. Its plasma concentration increases with nutrient stimuli.

The first reported biologic action of amylin was the inhibition of insulin-stimulated incorporation of glucose into glycogen in isolated rat skeletal muscle [2]. This finding has been confirmed and extended in vitro [3] and in vivo and appears to result from activation of glycogen phosphorylase [4] and inhibition of glycogen synthase [5]. Amylin stimulation has been proposed [4] as underlying the previously unexplained increment in phosphorylase *a* activity observed after oral glucose [6]. It was proposed that amylin's actions could increase lactate production, and an early effect of amylin injections in intact rats is a rise in plasma lactate concentration [7]. Accordingly, we have suggested that amylin serves as a partner hormone with insulin [8] to promote lactate flux.

While some authors have concluded that plasma amylin is too low to have a significant endocrine function [9], several studies now report metabolic actions of exogenous amylin at reported plasma levels. However, those studies have not demonstrated metabolic regulation by endogenously secreted amylin. One approach to determining the effects of circulating amylin, and thereby whether it is behaving as a hormone, is to block its action with selective antagonists. This experimental test has been made possible by our having designed and synthesized a number of potent and selective amylin receptor antagonists, such as AC187, described here for the first time. A similar approach has been applied to the study of pancreatic islet function. Insulin secretion [10–14] and β-cell activity [15] were reported to be inhibited by amylin, but it was not clear whether endogenously secreted amylin had effects on β-cell secretion. Increased insulin secretion in the presence of amylin antagonists [16,17] support the idea [16] that endogenously secreted amylin reduces insulin secretion.

AC187 has been shown to have appropriate pharmacologic properties in binding studies and in isolated tissues. AC187 competes potently for rat amylin binding at high affinity sites such as rat nucleus accumbens membranes [18] which have been useful in developing a number of selective ligands, including AC187. The K_d for amylin at this site is 28 pM, and the K_i for AC187 is 79 pM. AC187 is relatively selective in competing for amylin binding, displacing amylin from nucleus accumbens membranes with over 400-fold greater potency than it

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displaces [^{125}I]hCGRP from CGRP receptors (SK-N-MC cells [19]), and with 38-fold greater potency than that with which it displaces [^{125}I]salmon calcitonin from calcitonin receptors (T47D cells [20]). Second, when present at 30 nM, AC187 reverses the inhibitory action of 100 nM rat amylin on insulin-stimulated incorporation of [U-^{14}C]glucose into glycogen in isolated rat soleus muscle [3] by 50%.

The first part of the present study describes the ability of AC187 to block the metabolic actions of exogenous amylin in vivo. Using this amylin antagonist, we then tested whether endogenously secreted amylin causes part of the increase in plasma lactate seen after a glucose load. When glucose and insulin profiles were matched, preinfusion of AC187 reduced the surge in plasma lactate following glucose administration, indicating that the processes responsible for it were sensitive to normally circulating levels of amylin.

2. Materials and methods

2.1. Animals

Male Harlan Sprague–Dawley rats (weight 250–350 g) were used in all studies. In all preparations, rats were anaesthetized with halothane, tracheotomized, and cannulated via the femoral artery and saphenous vein. A 2-h stabilization period under light anaesthesia then followed to allow metabolic stabilization of the preparation before recordings began.

2.2. AC187

AC187 (amino acid sequence *N*-acetyl-Val-Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Asn-Thr-Tyr-NH_2, MW 2890) was made in house as the TFA salt by solid phase peptide synthesis and purified by reverse-phase high-performance liquid chromatography to a purity of 99%. Peptide content was 74.6 ± 1.4%.

2.3. In vivo characterization of AC187

Animals fasted for 18–20 h were anesthetized and prepared as above before intravenous preinfusion with either AC187 (1.5 μmol/kg, then 5 nmol/kg/min) or saline from $t = -30$ min until $t = +60$ min. This was followed by an intravenous infusion of either rat amylin (36 nmol/kg, then 0.6 nmol/kg/min) or saline which began 30 min later at $t = 0$ min and continued throughout the experiment. There were, therefore, 4 treatment groups: saline + saline ($n = 7$), saline + amylin ($n = 8$), AC187 + saline ($n = 3$), and AC187 + amylin ($n = 4$).

2.4. Lactate responses following intravenous glucose

Fed male Harlan Sprague–Dawley rats (360–420 g) were anesthetized and prepared as described above before intravenous administration of 5.2 mmol/kg (2 mmol) D-glucose over 2 min. Arterial samples were taken at the times indicated and plasma immediately analyzed by immobilized enzyme chemistries (YSI 2300 STAT analyzer, Yellow Springs, OH) for glucose and lactate. Additional samples were frozen and subsequently analyzed by radioimmunoassay (RIA kit 06130, INCStar Corp, MN) for insulin. One group of rats ($n = 18$) was administered the amylin antagonist AC187 as a primed/continuous infusion (131 nmol/kg + 4.4 nmol/kg/min) from $t = -10$ to $t = +120$ min to block the actions of endogenous amylin. Control rats ($n = 27$) received intravenous glucose, but saline instead of AC187. Another group of rats ($n = 8$) was administered 0.1 nmol insulin (Humulin-R, Eli Lilly, Indianapolis) by subcutaneous injection 3 min after the i.v. glucose load so that the plasma insulin profile would match that of the AC187-treated group. This control group was required because insulin secretion is inhibited by amylin and enhanced by amylin blockers such as $^{8-37}$hCGRP [16], $^{8-32}$sCalcitonin (Marco et al., unpublished) and $^{8-37}$rAmylin [17]. Lactate responses were integrated by the trapezoidal integral method for the 60 min following glucose administration and expressed as increments above the values obtained in rats not administered glucose ($n = 5$).

3. Results

3.1. In vivo characterization of AC187

In the present in vivo study, AC187 was able to inhibit metabolic responses to exogenous amylin in the intact animal. Fig. 1 shows that an infusion of AC187 that was itself without effect on lactate levels in anesthetized rats, inhibited by more than 90% the lactate increment evoked by infusions of rat amylin. When the AC187 infusion was stopped but the amylin continued, the response typical of amylin agonists (an increase in lactate) returned, indicating reversibility of the antagonism. Other experiments (data not shown) have shown that AC187-induced blockade of amylin action is surmountable by higher doses of amylin, indicative of a competitive (surmountable) antagonism.

Other experiments indicate that the infusion regimen for AC187 used in the present preparation could be expected to suppress the response of any endogenously secreted amylin. The 131 nmol/kg + 4.4 nmol/kg/min AC187 infusions used glucose challenge experiments of the present study were able to block the lactemic effects of 10 μg exogenous amylin injected subcutaneously. The incremental area under the 0 → 60 min lactate curve was 37.7 ± 3.8 mM · min compared to 52.4 ± 3.8 mM · min

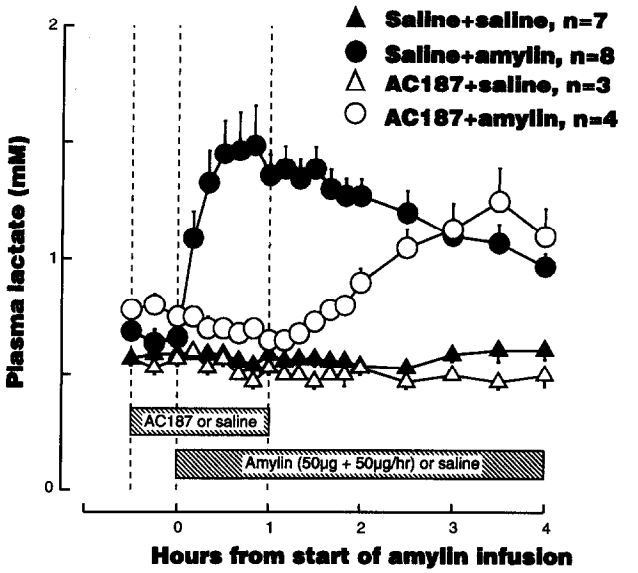

Fig. 1. Blockade of amylin-induced hyperlactemia by AC187. Arterial plasma lactate increased in rats receiving saline then amylin. Rats receiving AC187 then amylin showed no change in plasma lactate, a response similar to saline + saline infused controls and similar to rats receiving AC187 + vehicle alone. In rats receiving AC187 + amylin, the agonist response (an increase in lactate) returned when infusion of the antagonist was stopped. Symbols are means ± S.E.M.

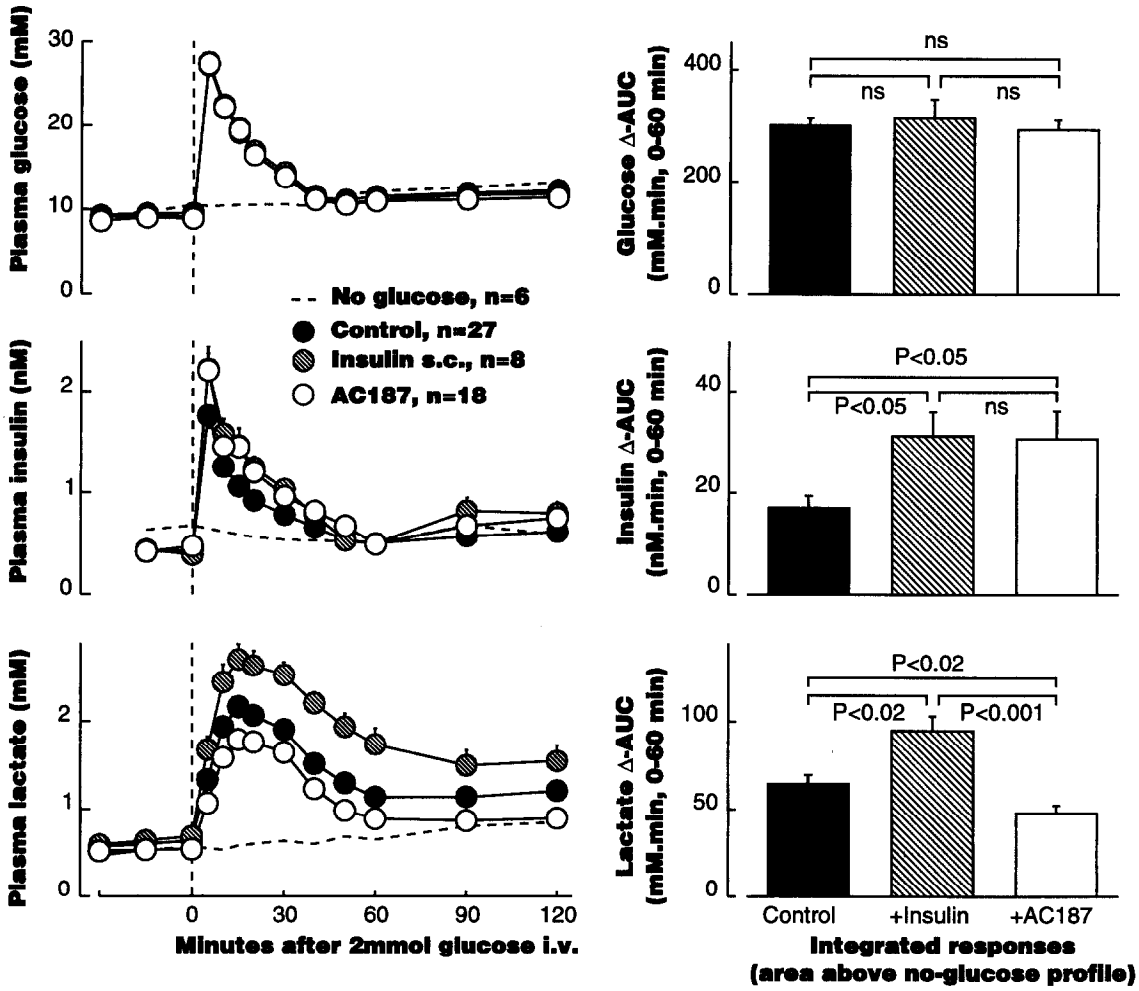

Fig. 2. Glucose, insulin and lactate concentrations following intravenous glucose administration into control, insulin-injected and AC 187-infused rats. Plasma concntrations are shown in the left-hand graphs. Integrated responses, which are the area between the concentration profiles of the glucose-infused animals and the no-glucose (baseline) group between 0 and 60 minutes after glucose, are plotted as bars in the right-hand graphs. Changes in plasma glucose were equivalent in all groups, as shown by the upper bar graph. The lactate surge after glucose was increased over control values (lower panels; $P < 0.02$) when the plasma insulin profile was augmented by a subcutaneous injection. When the amylin antagonist AC187 was administered to block actions of endogenous amylin, the insulin response was 81% greater than the control response (middle panels; $P < 0.05$), consistent with previous interpretations that endogenous amylin tonically inhibits insulin release. The lactate response (lower panels) in AC187-infused animals was depressed not only below the levels observed in insulin-injected rats with matched glucose and insulin profiles ($P < 0.001$), but also below those in control rats ($P < 0.02$). Symbols are means ± S.E.M. The significance of differences between bars is indicated next to the relevant ties between bars.

with 10 μg amylin alone ($P < 0.05$), and was similar to responses observed with saline or AC187 only (34.4 ± 1.9 and 35.1 ± 2.6, respectively; n.s.). Such 10 μg amylin injections produce peak plasma levels of 347 pM, which exceeds endogenous amylin levels observed in normal Sprague–Dawley rats.

3.2. Lactate responses following intravenous glucose

Figure 2 shows plasma glucose, lactate and insulin concentrations before and after an intravenous administration of 2 mmol glucose alone. As expected, glucose rose almost immediately to a peak and then declined towards baseline levels. Insulin levels rose for about 15 min and then declined over the next 2 h. As seen in the lower panel, lactate levels approximately doubled over the first 30 min and then slowly declined.

The same experiment was performed with endogenous insulin release supplemented by a subcutaneous injection of 0.1 nmol insulin. This dose and mode of administration increased the 0 → 60 min incremental insulin response by 84%, an amount that was designed to closely mimic ($P = 0.96$) the 81% increase in insulin response observed in the presence of amylin blockade by AC187. Following such insulin supplementation, the lactate surge was 47% higher than that seen under control conditions.

Also shown in Fig. 2 are the responses to intravenous glucose under conditions where AC187 was used to

block the action of endogenous amylin. The change in plasma glucose was similar to that seen under controlled conditions. The incremental insulin response was some 81% greater than that observed under control conditions ($P < 0.02$), consistent with results obtained from other studies [16,17] where amylin antagonists increased nutrient-stimulated insulin secretion, as described in section 1.

The lactate response are shown as shaded and open symbols in Fig. 2. The lactate response (change in area under the curve) in the presence of AC187 was only half of that seen when insulin levels were matched by supplemental s.c. injections ($P < 0.001$), and was in fact less than that seen under control conditions where insulin was lower ($P < 0.02$).

4. Discussion

The observation that added insulin increased lactate levels after glucose is consistent with the conventional view [21] that increased lactate generation is the result increased glycolytic flux, driven by both the increased glucose gradient and increases in insulin-mediated glucose transport.

However, from this view one would also have predicted that similar glucose and insulin profiles would result in similar lactate profiles. That is, with equivalent increases in insulin-mediated transport and in the gradient driving glucose into cells, increases in non-oxidative glycolytic flux and lactate generation should also have been equivalent.

In the present experiments, the glucose profiles and insulin profiles were well matched in the group of animals injected with extra insulin and in the group treated with the amylin antagonist. In spite of this matching, the lactate response in the AC187-infused rats was only half of that observed in the insulin-injected group.

These observations are consistent with AC187 having blocked the actions of a lactate-mobilizing agent that is released in response to glucose. The most likely explanation is that we have observed the effects of blocking endogenously released amylin.

A conceivable alternative interpretation of the present data is that AC187 could have blocked a naturally-occurring ligand other than amylin which was increased in response to glucose administration. Some authors [22] have proposed that calcitonin gene-related peptide (CGRP) may be involved in regulating carbohydrate metabolism with amylin-like actions. How, then, can we be confident that it is not the effects of endogenous CGRP which are being blocked? First, CGRP levels have been reported not to increase in plasma after glucose administration [23]. Second, the lack of any change in blood pressure in our experiments makes it unlikely that CGRP, at least systemically, was increased, since CGRP's most powerful action is on vasodilation. Third, AC187 is highly selective for amylin action over CGRP action on skeletal muscle. It has recently been determined (Irsula et al., unpublished) that AC187 is 500-fold less potent in blocking the effects of CGRP on skeletal muscle than it is at blocking the effects of amylin on skeletal muscle. Because the lactate response was indeed blockable by AC187, the present observations are unlikely to result from the effects of endogenous CGRP. Thus, the data are most easily explained by the amylin receptor antagonist having blocked release of lactate stimulated by endogenous amylin.

The source of lactate following a glucose load has been controversial. Candidate tissues have included those where glucose metabolism is insulin-insensitive, such as gut [24], as well as those where it is insulin-sensitive (fat, muscle and liver). A recent detailed study in conscious dogs [25] indicated that much of the lactate rise following glucose absorption appeared to come from stored carbohydrate, predicted to be muscle glycogen, and not from the absorbed glucose. While the techniques in the present study could not have determined the tissue sources of lactate, it is likely that they included insulin-sensitive tissues, since addition of insulin increased the lactate response. The amylin blockade experiments reported here, now also indicate that up to half of the lactate response may be derived from amylin-sensitive tissue, which includes muscle, but not fat [26].

The use of selective amylin blockade is a useful tool in the definition of the role of endogenous amylin. The present findings obtained using such an agent represent the first demonstration in normal animals of an endocrine action of endogenous amylin that was distinct from an autocrine role at the pancreas, and therefore support an hormonal role.

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