Rapid communication

Amylin tonally regulates arginine-stimulated insulin secretion in rats

W. M. Bennet¹, C. S. Beis¹, M. A. Ghatel¹, P. G. H. Byfield², S. R. Bloom¹

¹ Department of Medicine, Royal Postgraduate Medical School, London, UK

² Haemostasis Research Group, Clinical Research Centre, Harrow, UK

Summary To assess the effect of naturally-present amylin in the control of insulin release we infused a novel amylin antagonist, its 8–37 fragment, or amylin in anaesthetized rats for 60 min, and 30 min after the start arginine was infused for 14 min. Amylin_{8–37} decreased the blood glucose concentration by 18% whereas the plasma insulin concentration was 90% higher following arginine treatment. In contrast amylin infusion

raised both glucose and insulin concentrations. These results suggest that while amylin added at high dose can induce peripheral insulin resistance, naturally present amylin tonally controls insulin secretion. [Diabetologia (1994) 37: 436–438]

Key words Amylin physiology, insulin secretion, amylin antagonists, islet paracrine physiology.

Amylin (islet amyloid polypeptide) is a 37-amino acid polypeptide synthesised in the islet beta cell [1]. Circulating plasma concentrations are of the order 2–20 pmol/l, whereas systemic biological actions of the peptide are evident at nanomolar concentrations [2, 3]. Reported extra-islet effects of amylin infusion in animals may represent ‘cross-talk’ at receptors for the homologous peptides CGRP and calcitonin [4]. As amylin is secreted from the beta cell into an islet portal circulation [5], the local intra-islet concentrations are greater than in the peripheral circulation. This may be sufficient to blunt insulin secretion, an effect demonstrated to occur at a plasma concentration of approximately 2 nmol/l in human subjects [6] and at 75 pmol/l in isolated perfused rat pancreas [7].

The development of competitive antagonists of amylin may provide a tool to separate its physiologic from pharmacologic actions. The 8–37 fragment of

amylin competitively displaces [¹²⁵I]amylin from lung membranes [4], and inhibits the effects of added amylin to depress insulin secretion and increases insulin secretion from perfused, isolated rat islets [8]. CGRP_{8–37} has been shown to enhance arginine-stimulated insulin secretion from anaesthetized rats [9], although it is not clear if this peptide antagonist predominately acts by attenuating CGRP effects or amylin effects in the islet. We have therefore tested whether amylin_{8–37} could enhance insulin secretion in rats in vivo.

Materials and methods

Animals and treatment

Male Wistar rats (200–250 g, $n = 27$) were obtained from Bantin and Kingman (Hull, UK) and maintained on rat feed ad libitum. They were fasted from 16.00 hours on the day prior to study. On the study day general anaesthesia was induced with an intraperitoneal injection of 0.7 ml of an aqueous mixture of 1.25 mg/ml midazolam (Hypnovel, Roche, Welwyn Garden City, UK), 91 µg/ml fentanyl citrate and 2.5 mg/ml fluanisone (Hypnorm, Janssen, Oxford, UK), and maintained with further 0.25 ml doses. Cannulae (0.63 mm outer diameter; Portex, Hythe, UK) were placed in a femoral vein for infusion of test substances and in a femoral artery for blood sampling, and were kept patent with flushes of 0.9% NaCl (saline) containing 50 units/ml heparin. At time 0 min a primed, continuous infusion of amylin_{8–37} or amylin

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Corresponding author: Professor S. R. Bloom, Department of Medicine, Royal Postgraduate Medical School, Du Cane Road, London W12 0NN, UK

Abbreviations: CGRP, Calcitonin gene-related peptide.

Fig. 1A, B. Blood glucose (A) and plasma insulin (B) concentrations in rats during and after an infusion of either amylin₈₋₃₇ (●) or amylin (■) or saline control (○). Arginine (ARG) was infused from 30–44 min. Results are mean $\pm$ SEM, calculated with log data for insulin. Compared to respective control rat values, blood glucose concentrations were lower at 20 and 80 min with amylin₈₋₃₇ infusion, and were higher at all time points during amylin infusion. Plasma insulin concentration was raised at 60 min during amylin₈₋₃₇ infusion and at 30, 60 and 90 min during amylin infusion

(0.1 mg, 0.4 mg/h) was commenced and continued for 60 min, and 10% rat plasma was given to control animals. At 30 min arginine (2 mol/l) was infused for 14 min at 50 μ l/min, a rate which is insufficient to alter blood pressure. Blood samples were taken at 10, 20, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90 and 120 min for measurement of the blood glucose concentration using Glucostix (Ames, Basingstoke, UK) and a Glucometer II (Ames), and at 30, 60, 90 and 120 min for measurement of the plasma insulin concentration by RIA [10].

Preparation of peptides

Rat amylin amide was custom synthesised (Professor B. Penke, Department of Medical Chemistry, Albert Szent-Györgyi Medical University, Szeged, Hungary). We synthesised amidated rat amylin₈₋₃₇ using 9-fluorenylmethoxycarbonyl chemistry on an automated synthesizer (Model 431 A; Applied Biosystems, Warrington, UK) [8]. Peptides were purified with HPLC and identities confirmed by mass spectrometry, amino acid analysis, and receptor binding studies [4]. They were prepared immediately before use as 0.2 mg/ml solutions in saline containing 10% rat plasma.

Statistical analysis

Data are presented as means and SEM with the exception of insulin where the geometric means and 95% confidence limits are shown as the data approximated to a log-normal distribution. Statistical analysis was by repeated-measures analysis of variation with subsequent comparisons by linear contrast, using log data for insulin. A *p* value of less than 0.05 was considered to be statistically significant.

Results

The rat body weights were similar in each study group (control: 250 ± 13 g, $n = 12$; amylin₈₋₃₇: 251 ± 11 g, $n = 9$; amylin: 253 ± 10 g, $n = 6$). In the control group the blood glucose concentration was initially stable, but increased following arginine infusion, and progressively rose after the vehicle infusion was terminated at 60 min (Fig. 1A). In the amylin₈₋₃₇-infused rats there was an initial decrease in blood glucose concentration to a value at 20 min that was 14% lower ($F_{1,24} = 5.56$, $p = 0.03$) than for control rats. After arginine infusion at 45 min the blood glucose level tended to remain lower by 13% ($p > 0.1$). At 20 min after discontinuation of the amylin₈₋₃₇ infusion, the blood glucose concentration was lower by 18% ($F_{1,24} = 4.45$, $p = 0.04$) in the amylin₈₋₃₇-infused rats compared to control animals, after which values in the antagonist-treated group increased to match those in the control group. When amylin was infused the blood glucose concentration was markedly higher ($p < 0.05$) than for control rats at all time points. Plasma insulin concentration in control animals was 48.4 pmol/l (95% confidence limits 22–109) after 30 min of vehicle infusion prior to arginine administration (Fig. 1B). The insulin concentration was raised by a mean of 50% at the same time point during amylin₈₋₃₇ infusion but the difference was not statistically significant (74, 26–203 pmol/l, $p > 0.1$). The arginine infusion increased the plasma insulin concentration in control rats to 133, 85–209 pmol/l, with the corresponding value in amylin₈₋₃₇ infused rats found to be two-fold higher (252, 179–355 pmol/l, $p = 0.02$). At 30 and 60 min after the amylin₈₋₃₇ infusion, the plasma insulin concentrations matched those in control rats. During amylin infusion plasma insulin concentrations were higher than for control rats before ($p < 0.05$) and following ($p < 0.001$) arginine administration, and 30 min after amylin infusion ($p = 0.05$).

Discussion

This study demonstrates for the first time that plasma insulin concentrations can be raised in vivo when the tonal effects of endogenous amylin were blocked with the antagonist, amylin₈₋₃₇. This effect occurred despite a related lowering of the blood glucose concentration which implies that enhanced insulin secretion was the

seminal effect of amylin antagonism. We observed a maximal hypoglycaemic response to amylin₈₋₃₇ 20 min after the peptide infusion was discontinued which temporally followed the peak insulin secretion. Young et al. [9] reported a glucose nadir at a similar time point after CGRP₈₋₃₇ infusion in fed rats studied with a similar protocol. Our result implies that endogenous amylin is an autocrine or paracrine tonal regulator of insulin release. Infusion of the complete peptide induced hyperglycaemia and hyperinsulinaemia, but at a body weight-normalised infusion rate which was two orders of magnitude higher than previously tested in human subjects [6]. This effect on glucose homeostasis is in direct contrast to the effect of amylin₈₋₃₇, and it indicates that the seminal effect of high-dose amylin is an elevation of glucose concentration, by means of increasing insulin resistance, with subsequent enhancement of insulin secretion. Our results provide suggestive evidence that endogenous amylin is a physiological tonal regulator of insulin secretion, whereas insulin resistance is merely a *pharmacologic* action of the peptide. Amylin may be implicated in the development of human diabetes mellitus by impairing insulin secretion [3, 6–8].

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