Reduced Insulin Secretion and Content in VEGF-A Deficient Mouse Pancreatic Islets

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Abstract

Mice, deficient for vascular endothelial growth factor VEGF-A in pancreatic islets, have reduced insulin gene expression levels and an impaired glucose tolerance. Here, we investigated whether VEGF-A was required for physiological glucose-stimulated insulin secretion and insulin content. We performed in situ pancreas perfusions and islet perifusions on mice lacking VEGF-A in the pancreatic epithelium in order to study their ability to secrete insulin in response to glucose. We identified insulin secretion defects in the pancreata of VEGF-A deficient mice, including a delayed and blunted response to glucose. Islet perifusion experiments revealed a missing first phase and weaker second phase of insulin secretion, in two of three VEGF-A deficient mice. On average, insulin content in VEGF-A deficient islets was significantly reduced when compared with control islets. We conclude that VEGF-A is required in pancreatic islets for normal glucose-stimulated insulin secretion and physiological insulin content. Thus, VEGF-A is a key factor for pancreatic islet function.

Abbreviations

VEGF-A	Vascular endothelial growth factor A
VEGFR2	VEGF-A receptor 2
VEGF KO	*Pdx1-Cre x VEGF-A loxP* mouse

Introduction

Pancreatic islets express high levels of vascular endothelial growth factor, VEGF-A, throughout life. VEGF-A establishes and maintains a dense capillary network inside islets by signalling to VEGFR2-expressing endothelial cells [1–3]. A physiological requirement for islet VEGF-A is revealed by a glucose tolerance defect in mice lacking VEGF-A (VEGF KO) [3]. Interestingly, overexpression of VEGF-A in mouse pancreatic islets improves their insulin content as well as success of subsequent islet transplantation [4, 5]. Moreover, decreased insulin secretion was observed in STAT3-deficient mouse pancreatic islets, coinciding with a reduced VEGF-A expression in these islets [6].

These findings raise the question whether the VEGF-A induced capillary network affects glucose-stimulated insulin secretion. We therefore analyzed the physiological role of VEGF-A in insulin secretion.

Islets deficient for VEGF-A were recently demonstrated to express lower insulin mRNA levels [7]. Since posttranscriptional regulation was shown to affect insulin content and secretory granule biogenesis [8], we also explored whether VEGF-A was required for physiological insulin protein content.

Materials and Methods

Mice

VEGF KO mice (Pdx1-Cre x VEGFloxP) expressed Cre recombinase under the Pdx1 promoter and had both VEGF-A alleles floxed [3]. Littermates with no homozygous VEGF-A deletion were used as controls. Mice were kept in accordance with the German and Swiss law and the NIH principles of laboratory animal care.

In situ pancreas perfusion

Mice were anesthetized and prepared as previously described [9]. In brief, the pancreas was perfused at 37 °C with a basal solution (KRB containing 10 mmol/l leucine, 0.25% fraction V BSA

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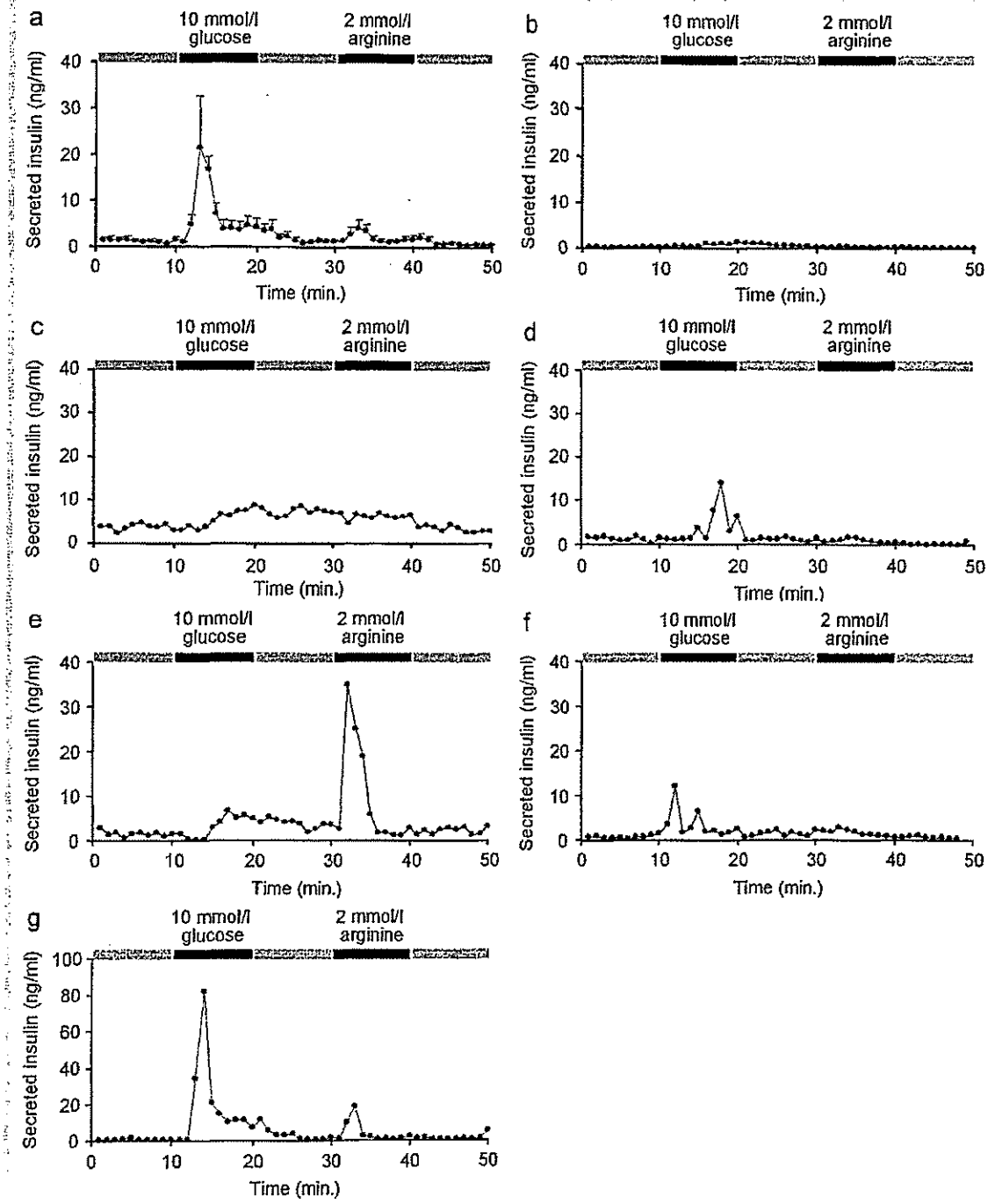

Fig. 1 Kinetics of glucose and arginine stimulated insulin secretion during in situ pancreas perfusions. Mice were perfused with each solution for 10 minutes, and the effluent was collected every minute, starting with basal solution (grey bar), followed by 10 mmol/l glucose (black bar), then basal solution, next 2 mmol/l arginine (black bar), and finally basal solution. **a** Insulin secretion per minute was presented as means ± SEM of 5 control mice to be compared with **(b-g)** 6 single VEGF KO mice.

and 40 g/l dextran), supplemented with 2.8 mmol/l glucose, 10 mmol/l glucose or 2 mmol/l arginine, at a flow rate of 1.5 ml/min. After 30 min perfusion with basal solution containing 2.8 mmol/l glucose, the effluent was collected in 1-min intervals from a catheter placed in the portal vein. The insulin content of each fraction was determined by RIA.

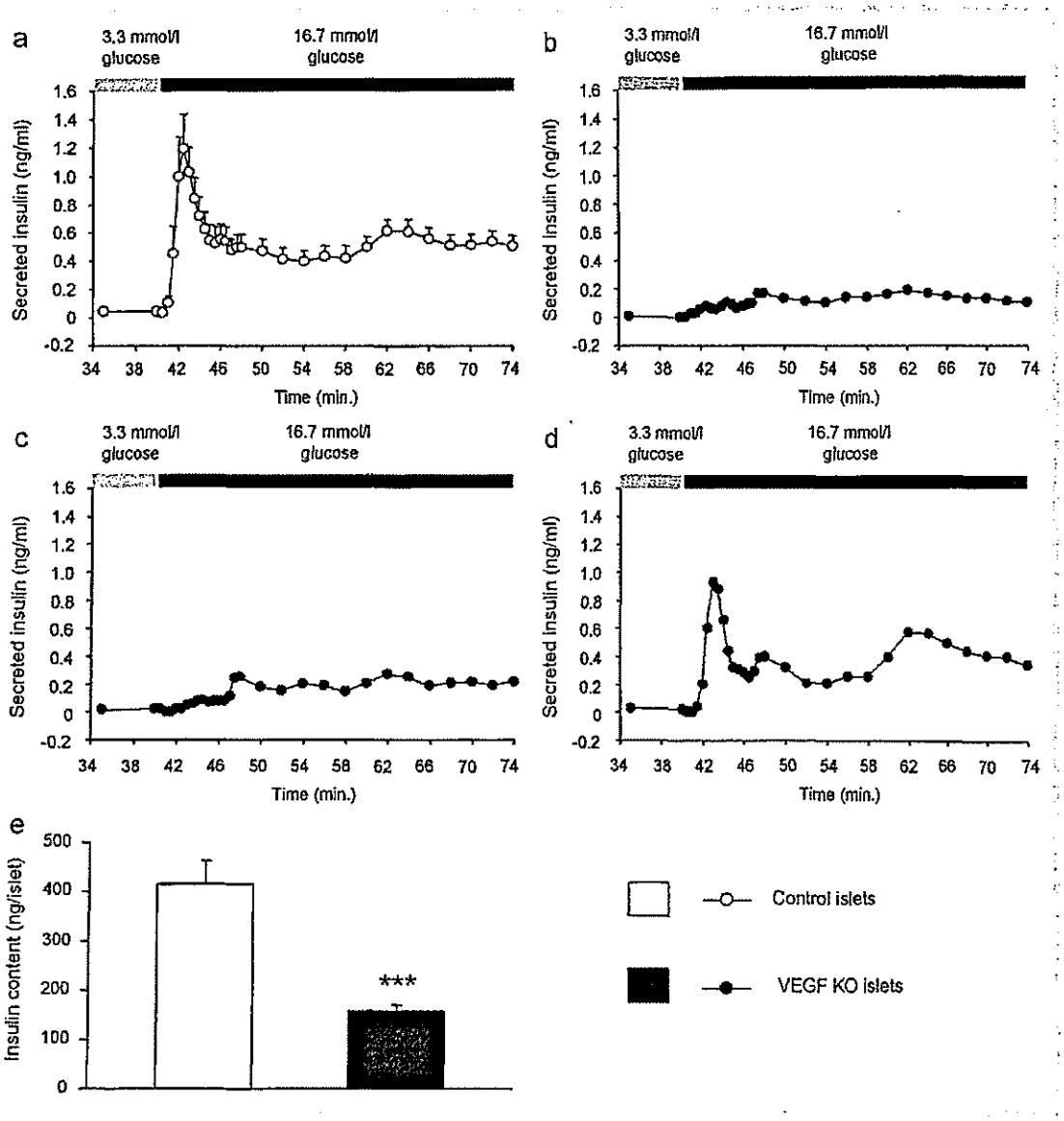

Fig. 2 Insulin kinetics of glucose stimulated insulin secretion during pancreatic islet perifusions, and insulin content. a–d Secreted insulin in the effluent of pancreatic islets perifused with 3.3 mmol/l glucose and stimulatory 16.7 mmol/l glucose concentrations. a Secreted insulin of islets isolated from 3 control mice (1–2 sets of islets per mouse). Data are means ± SEM. b–d Insulin secreted by islets isolated from 3 VEGF KO mice (1 set of islets per mouse). e Insulin content in pancreatic islets isolated from 3 control mice (white bar) and 3 VEGF KO mice (black bar). Values are means ± SEM. ***p<0.001 (Student's t-test).

Islet perifusion experiments

VEGF KO and control islets were simultaneously perifused at 37°C in a 12-channel perifusion system [10] with KRB containing (*in mmol/l*) 2.5 $CaCl_2$, 10.0 HEPES, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 123.0 NaCl, 20.2 $NaHCO_3$ and 0.2% bovine serum albumin. During a 40-min starvation period, the mouse islets were perifused with the KRB supplemented with 3.3 mmol/l glucose. For stimulation, the glucose concentration was raised to 16.7 mmol/l. Effluent was collected in intervals of 5 min during starvation period, 0.5 min during first phase and 2 min during second phase. Insulin, secreted by 15 islets per channel, was measured by RIA.

Insulin content

Islets were isolated from 3 VEGF KO and 3 control mice. Groups of 15 similar sized islets (3–4 observations/mouse) were lysed, and the insulin content was determined as previously described [10].

Results

We performed in situ pancreas perfusions on six VEGF KO mice and their control littermates. On average, glucose stimulated insulin secretion was biphasic in control mice (○ Fig. 1a). In contrast, insulin secretion in VEGF KO mice was variable and

therefore presented individually for each mouse (○ Fig. 1b–g). Normal biphasic insulin secretion was lost in four of the six mice (○ Fig. 1b–e) and glucose stimulated insulin secretion was attenuated and/or delayed in all but one case (○ Fig. 1g). In this case, the mouse had a normal pancreas rather than the pale and small pancreas characteristic of a VEGF-A deletion. Interestingly, along with the missing VEGF KO phenotype, this mouse had a normal biphasic insulin secretion (○ Fig. 1g). In addition to the defects in glucose-stimulated insulin secretion, we also observed that the arginine response was largely undetectable in four of the six mutant mice (○ Fig. 1b,c,d,f).

Islet perifusions were performed with non-stimulatory (3.3 mmol/l) and stimulatory (16.7 mmol/l) glucose concentrations (○ Fig. 2a–d). In these experiments, insulin secretion of similar sized VEGF KO and control islets was measured simultaneously in a 12-channel perifusion system [10]. Glucose stimulated insulin secretion was biphasic in 5 islet preparations of 3 control mice (○ Fig. 2a, merged graph). In contrast, perifusion experiments with islets from VEGF KO mice (two of three), revealed a missing first phase of insulin secretion (○ Fig. 2b,c), whereas the second phase was detectable (○ Fig. 2b,c). Similar to the in situ pancreas perfusion experiments, we identified one VEGF KO mouse with a normal biphasic insulin secretion (○ Fig. 2d).

Finally, islets of a similar size were isolated from VEGF KO mice and control littermates to measure their insulin content. As shown in ○ Fig. 2e, VEGF-A deficient islets contained 2.6-fold less insulin when compared with control islets.

Discussion

VEGF-A is required for development and maintenance of the particularly dense capillary network characteristic of pancreatic islets [1–3]. This capillary network is necessary for normal glucose tolerance of mice [3], raising the question whether the VEGF-A induced capillary network is required for the ability of pancreatic islets to secrete insulin. Here we identified insulin secretion defects in VEGF KO mice by using two independent methods, in situ pancreas perfusion and islet perifusion. Both methods revealed either a loss of and/or a delay in first phase insulin secretion in most VEGF KO mice.

At first glance, our findings seem to contradict a recently published study that showed an improved glucose tolerance in mice treated with VEGF inhibitors [1]. In these mice, many capillaries regressed from the pancreatic islets. However, in contrast to the VEGF KO islets, their vascular basement membranes remained within the islets [1]. Therefore, this difference in the vascular basement membrane might explain the different results in the glucose tolerance of VEGF KO mice and VEGF inhibitor treated mice.

Our recent study supports this interpretation. We have shown that capillary endothelial cells provide beta cells with a vascular basement membrane and that this basement membrane is needed for physiological insulin gene expression levels [7]. Because gene expression does not necessarily correlate with protein expression, due to posttranscriptional regulation [8], we addressed the question whether the VEGF-A induced capillary network also affects total insulin content of pancreatic islets. Our results show that posttranscriptional regulation does not compensate for decreased insulin gene expression in the absence of VEGF-A. Instead, insulin content is reduced to a similar extent as insulin gene expression.

In summary, our data demonstrate that the VEGF-A induced capillary network is required *in vivo* for pancreatic beta cell function at the levels of insulin gene expression, insulin content and insulin secretion.

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