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SHORT COMMUNICATION

A novel role for vitamin D: modulation of expression and function of the local renin–angiotensin system in mouse pancreatic islets

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Abstract
Aims/hypothesis The aim of this study was to demonstrate that hormonal vitamin D (calcitriol) modulates the local pancreatic islet renin-angiotensin system (RAS) whilst improving islet beta cell secretory function.
Methods Isolated islets cultured ex vivo under high- or low-glucose conditions and treated with or without calcitriol were examined for changes in RAS component activity and glucose-stimulated insulin secretion (GSIS). Isolated islets from vitamin D receptor knockout (VDR-KO) mice were compared with islets from wild-type (WT) mice for major RAS component expression and RAS protein production.
Results Isolated islets incubated ex vivo under high-glucose conditions showed increased expression and production of major RAS components; this was prevented and reversed by calcitriol in parallel with increases in GSIS. VDR-KO mice displayed increased RAS component mRNA expression and protein production as compared with WT mice, despite comparable glucose homeostasis.
Conclusions Young mice with vitamin D receptor ablation showed abnormal increases in islet RAS components at mRNA and protein levels, despite unaltered glucose homeostasis. Calcitriol prevents and can correct induction of RAS component production under high-glucose conditions in parallel with the well-known effect of calcitriol on increasing islet beta cell secretory responses to glucose.

Keywords Angiotensin II · Calcitriol · Hyperglycaemia · Insulin secretion · Pancreatic islets · Type 2 diabetes · Vitamin D receptor

Abbreviations

AT_1 receptor	Angiotensin II type 1 receptor
GSIS	Glucose-stimulated insulin secretion
$25(OH)D_3$	25-Hydroxycholecalciferol
IPGTT	Intra-peritoneal glucose tolerance test
RAS	Renin–angiotensin system
VDR	Vitamin D receptor
VDR-KO	*Vdr*-knockout
WT	Wild-type

Introduction

Convergent data have demonstrated functional renin-angiotensin systems (RASs) locally in many tissues and organs, with various physiological roles. Concurrently, we have demonstrated local RAS expression in pancreatic islets, which varies with glycaemic control [1] and is excessively expressed in a mouse model of type 2 diabetes [2]; angiotensin II type 1 receptor (AT_1 receptor) blockade improves islet function and glucose tolerance in hyperglycaemia [2]. Hyperglycaemia may be worsened through AT_1 receptor-induced uncoupling protein 2-driven oxidative

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stress, reducing islet beta cell mass and insulin secretion [3]. The combination of AT_1 receptor blockers with other glucose-lowering agents has a synergistic effect in reducing type 2 diabetes risks [4], further indicating a critical role for the RAS in modulating pancreatic islet function.

Calcitriol-bound vitamin D receptors (VDRs) in human and rodent pancreatic islets have physiological roles in islet function, including insulin secretion. Clinical studies indicate relationships between vitamin D status, glycaemia and type 2 diabetes risks [5]; animal studies show decreased insulin secretory capacity and glycaemic control in vitamin D deficiency and in *Vdr* knockout (VDR-KO) mice [6]. Several clinical studies demonstrate inverse relationships between circulating 25-hydroxycholecalciferol [$25(OH)D_3$] and renin activity in hypertensive patients and with myocardial dysfunction, supported by animal VDR-KO data [6]. Vitamin D suppresses renin production, the rate-limiting enzyme in RAS activity [7]. We now report that activated hormonal vitamin D (calcitriol) suppresses expression and production of islet RAS components in isolated mouse pancreatic islets.

Methods

Animals Adult male C57BL/6J mice were obtained from the Laboratory Animal Services Centre of the Chinese University of Hong Kong with approval from the Animal Experimentation Ethics Committee of the Chinese University of Hong Kong (Ref. no. 08/049/ERG). Eight-week-old male VDR-KO mice and WT littermates were used as described previously [7] with ethical approval from the Institutional Animal Care and Use Committee at the University of Chicago.

Pancreatic islet isolation, primary culture and calcitriol pre-treatment As described previously [8], pancreatic islets isolated from adult C57BL/6J mice using intra-ductal collagenase injection methodology were cultured in medium containing different concentrations of glucose together with calcitriol (Alexi, Lausen, Switzerland) for 7 days.

Detection of mRNA and protein levels Isolated islets were processed for mRNA detection by conventional PCR and real-time quantitative PCR as described previously [4] using primer sequences listed in electronic supplementary material (ESM) Table 1, for western blotting after each experiment and islet beta cell immunofluorescence staining as previously described [3, 9] using antibodies listed in ESM Table 2.

Measurement of glucose homeostasis, glucose-stimulated insulin secretion (GSIS) and (pro)insulin biosynthesis Blood glucose concentrations and glucose tolerance were assessed as previously described [2]. Ten size-matched isolated islets from each experimental treatment group were used for measurement of GSIS and (pro)insulin biosynthesis as described previously [3, 8].

Statistics Results are expressed as means ± SEM. Data were analysed by two-tailed Student's *t* test, or one-way ANOVA, followed by Tukey's post hoc test.

Results

Effects of calcitriol on RAS component expression in isolated islets under high-glucose conditions Isolated islets were incubated under different glucose conditions together with or without continuous treatment with calcitriol (pre-treatment). Renin receptor and AT_1 receptor mRNA expression was clearly increased by incubation with 28 mmol/l glucose, while there were non-significant changes in angiotensinogen mRNA expression. Pre-treatment with 1×10^{-9} or 1×10^{-8} mol/l calcitriol prevented these increases although it did not induce significant changes in angiotensinogen expression (Fig. 1a–c). Inhibitory effects of calcitriol were also observed when calcitriol was added with high- but not low-glucose exposure, for renin, renin receptor and AT_1 receptor protein production (Fig. 1d–f). *Vdr* expression and VDR protein levels, shown in the islet beta cells (ESM Fig. 1a), increased with calcitriol treatment under both 28 and 5.6 mmol/l glucose conditions (ESM Fig. 1b–e). 1×10^{-9}mol/l calcitriol had no effects on RAS component mRNA or protein values in islets exposed to 5.6 mmol/l glucose (Fig. 1a–f). Post-treatment studies, where islets were treated with calcitriol after a 2 day 28 mmol/l glucose incubation, showed that the addition of calcitriol reversed high-glucose activation of RAS component expression and production to near normal levels, optimally, at 1×10^{-9} mol/l (ESM Fig. 2a–f).

Effects of calcitriol on GSIS under high-glucose stress in isolated islets Ten islets from each study group were incubated with 1.7 mmol/l glucose before challenge with 16.7 mmol/l glucose. Pre-treatment with 1×10^{-9} mol/l calcitriol (Fig. 1g) significantly improved glucose challenge-induced insulin secretion and similar effects were observed in high-glucose incubated islets after post-treatment with calcitriol (ESM Fig. 2g, h).

Glucose homeostasis and islet expression of major RAS components in VDR-KO mice VDR-KO mice had significantly lower body weights than their WT littermates at 8 weeks old (Fig. 2a). However, both fasting and random blood glucose concentrations in VDR-KO mice were indistinguishable from those of WT mice (Fig. 2b,c), as

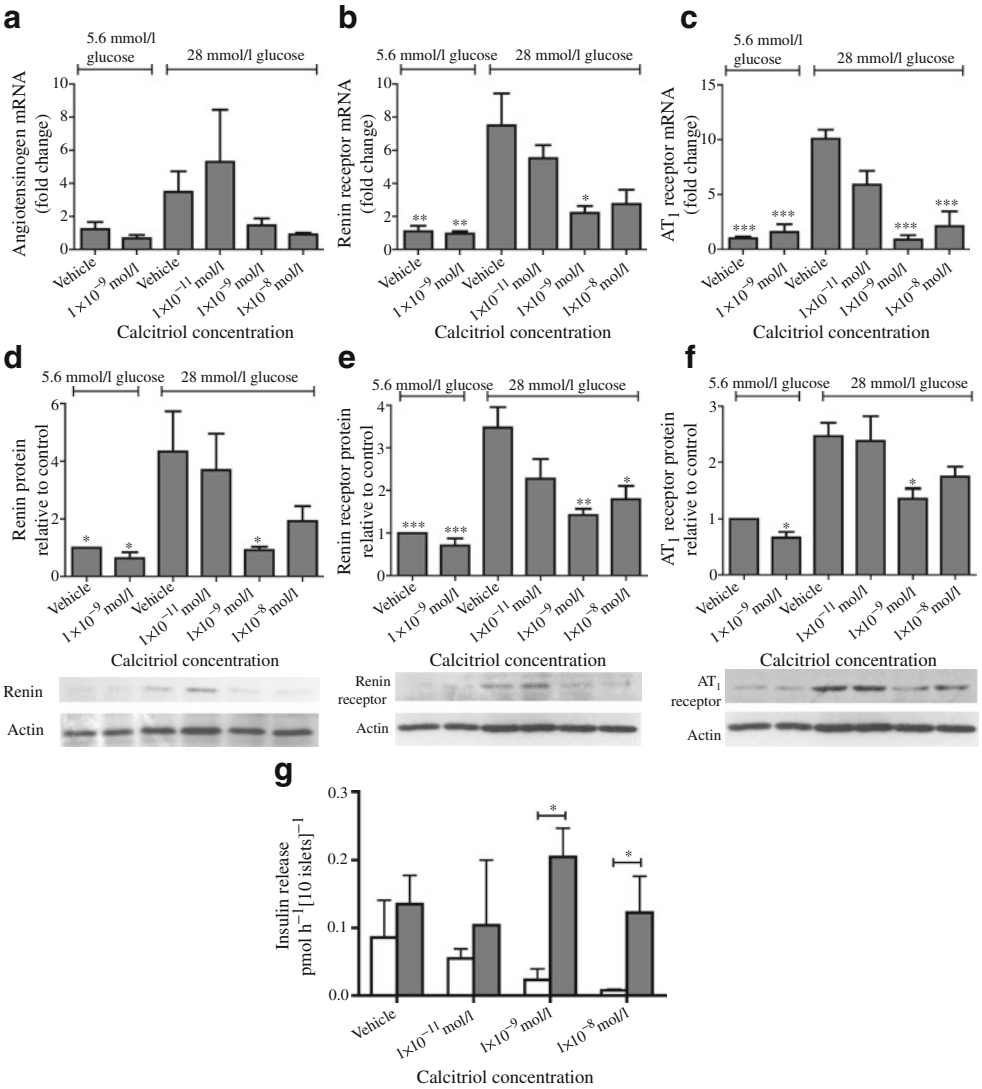

Fig. 1 The protective effect of pre-treatment of isolated islets with various concentrations of calcitriol for prevention of high-glucose-induced increases in RAS component expression and production, as well as induction of insulin secretion. Angiotensinogen (**a**), renin receptor (**b**) and AT_1 receptor (**c**) mRNA expression were measured in isolated islets pre-treated with calcitriol together with high or low concentrations of glucose (pre-treatment). These were assessed, together with protein production of renin (**d**), renin receptor (**e**) and AT_1 receptor (**f**), (*$p<0.05$, **$p<0.01$, ***$p<0.001$ vs 28 mmol/l glucose + vehicle; $n=6$). **g** Isolated islets incubated at 28 mmol/l glucose from experiments shown in (**d**–**f**) were examined for GSIS at both 1.7 mmol/l (white bar) and 16.7 mmol/l (grey bar) glucose, with/without added calcitriol (*$p<0.05$ vs 28 mmol/l glucose + vehicle; $n=7$)

reported previously [7]. Changes in glucose tolerance over time (IPGTT), expressed as AUC, did not differ between VDR-KO and WT mice (Fig. 2d,e). VDR-KO mouse islets exhibited increases in mRNA expression of, in particular, the renin receptor and AT_1 receptor (Fig. 2f–h) while renin protein was consistently and markedly increased in VDR-KO mouse islets (Fig. 2i).

Discussion

In the present study, we demonstrate, for the first time to our knowledge, that vitamin D modulates islet RAS components, identifying an additional mechanism for the beneficial effects of vitamin D on pancreatic islet beta cell function. The ablation of vitamin D effects in VDR-KO mice was associated with increased islet RAS component expression, independent of glucose concentration. High-glucose-induced increases in RAS components and GSIS were both protected, to various degrees, by prior exposure to physiological concentrations of hormonal vitamin D (calcitriol).

Various previous studies have suggested non-calcaemic roles of vitamin D, including beneficial effects on blood pressure, cardiovascular disease and diabetes risk, and elevated renin levels are reported in VDR-KO mouse kidney [7]. To determine the interaction of vitamin D with the islet RAS, we employed global VDR-KO mice, known to have elevated circulating renin and angiotensin II levels, as well as other local RAS components including renin [7]. However, glucose homeostasis in 8-week-old VDR-ablated mice was not abnormal, perhaps related to their smaller body size and other indirect effects of VDR-KO, although their islet size and structure were unchanged (Q. Cheng and P.S. Leung, unpublished data). Thus, changes in the islet RAS in 8-week-old mice appear to arise, not due to high glucose exposure but due to lack of ligand-bound VDR-induced suppression, since the changes are present even without hyperglycaemia. Therefore, physiologically, the

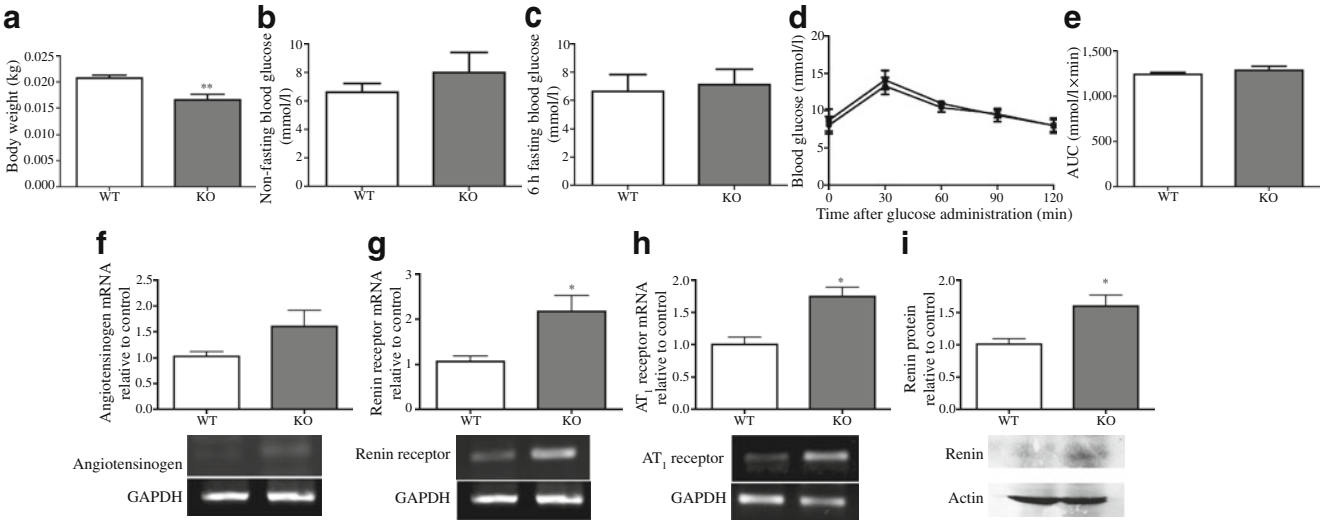

Fig. 2 Body weight, glucose homeostasis and pancreatic islet RAS component expression of VDR-KO mice and WT mice. (**a),** Body weight and (**b**), non-fasting and (**c**), fasting blood glucose concentrations were examined in 8-week-old VDR-KO and WT mice. IPGTT was measured using standard IPGTTs and glucose profile calculated as AUC (**d**, **e**). Major islet RAS component mRNA expression was measured by conventional PCR and semi-quantitative PCR (**f–h**), and renin protein production was assessed by western blot (**i**) (*$p<0.05$, **$p<0.01$ vs WT, $n=5$)

changes in the islet RAS in VDR-KO mice do not seem to be sufficient to alter glucose status or islet responses to insulin, although the induction of islet insulin release by pharmacological treatment with calcitriol reduced hyperglycaemia-related increases in local RAS activity in WT mice. Moreover, the lack of functional vitamin D signalling may activate compensatory effects in islets, which may affect local islet insulin signalling. More studies are required to understand the mechanisms relating vitamin D status to local RAS activity and to understand the effects of RAS activity on insulin release and glucose metabolism.

Abnormally high local pancreatic islet beta cell RAS component expression develops under hyperglycaemic conditions, both in vivo and in vitro [2, 8], and blockade of RAS activity has been proposed as a novel target for diabetes treatment [4]. In isolated islets in a continuously high-glucose environment together with calcitriol, abnormal RAS expression was inhibited whilst insulin secretion was enhanced, suggesting a potentially protective role of vitamin D for type 2 diabetes risk, which may be modulated via local RAS-related effects acting through the effects of the islet beta cell VDR on insulin secretory responses. This notion is supported by inverse relationships between baseline concentrations of $25(OH)D_3$ and type 2 diabetes risk, prospectively as well as cross-sectionally [10]. Thus, the maintenance of adequate vitamin D repletion over time could prove to contribute to diabetes avoidance at the population level, an effect that could be modulated, at least in part, through downregulation of islet RAS activity.

In conclusion, VDR-KO mice have increased islet RAS expression compared with WT mice, independent of blood glucose level. The increases in RAS component formation induced by high glucose concentrations in isolated mouse islets can be prevented by calcitriol, with concomitant increases in islet insulin secretion. Our findings, therefore, suggest protective effects of better vitamin D status against abnormal increases in islet RAS activity, especially in hyperglycaemia. These effects have the potential to contribute to islet beta cell protection and to reduction in type 2 diabetes risk, in addition to the well-known direct effects of calcitriol on insulin secretion. Further investigations are required to confirm a direct relationship between vitamin D and islet RAS, to determine the mechanisms by which vitamin D modulates islet RAS activity, to establish how increased RAS activity induces beta cell dysfunction in mice and in humans, and to determine whether RAS blockers and adequate provision of vitamin D can work synergistically for the reduction in type 2 diabetes risks.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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