in blood pressure rather than the decrease reported in several clinical studies. Thus, it appears plausible that vascular effects, which are a prerequisite for edema formation, may contribute to the overall effects of TZDs. Such effects may be due to interference with the angiotensinII/AT1R or endothelin-1 pathways, as well as an effect on the release of NO, all of which are regulated by PPARγ (Marx et al., 2004).

Which perspectives do these new and exciting results offer? First, since a direct role for PPARγ in kidney function has now been identified, the identification of its target genes will allow the initiation of genetic studies that may help identify individuals likely to develop edema. Similarly, if polymorphisms in the ENaC genes are associated with edema, patients at risk could be screened before initiating TZD therapy. Such genetic approaches should also provide evidence for the effects of TZDs in the kidney in humans. Second, the identification of the site of action of PPARγ in the kidney opens perspectives for targeted treatment with an appropriate diuretic in specific patients. Indeed, Guan et al. (2005) proposed that treatment with ameloride, a selective inhibitor of collecting duct salt absorption acting through ENaC, could be appropriate and provided experimental evidence for this. Further clinical studies should reveal whether diuretics acting on the distal nephron, such as ameloride or spironolactone, an inhibitor of aldosterone, will be effective in the treatment of TZD-induced edema in patients. Although by no means definitive, these data support the notion that TZDs act on the distal nephron. Third, further identification of the molecular mechanism of PPARγ in the renal collecting duct cells opens new perspectives for future drug development. Indeed, significant efforts are currently being undertaken to identify and characterize selective PPARγ modulators (SPPARMs) devoid of the undesirable side effects of the currently used TZDs. Although not unequivocally demonstrated, it is likely that the fluid retention effects of TZDs require higher doses of full agonists and thus partial PPARγ agonism may be another way to create an improved therapeutic window.

In conclusion, these recent reports clearly demonstrate a role for PPARγ in the kidney as a mechanism contributing to the blood volume expansion and possibly edema induced by TZDs. The information will undoubtedly contribute to the identification of patients at risk, their better management, and, possibly, the design of new, more selective drugs devoid of side effects. Depending on the outcome of the PROactive study, which will be the first of these landmark trials to report at the upcoming EASD meeting in September 2005, it can be expected that TZD use will be extended to prevent CVD in type 2 diabetic patients. Since edema is the principal clinical limitation for this class of drugs, further studies on the mechanisms involved are of major importance.

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Diabetic pancreatic β cells ARNT all they should be

A complex network of interacting transcription factors plays a critical role in normal pancreatic β cell function, with mutations in certain transcription factor genes known to cause diabetes. In a recent issue of *Cell*, Gunton et al. (2005) demonstrate a role for the transcription factor ARNT/HIF1β (hydrocarbon nuclear receptor translocator/hypoxia-inducible factor 1 β) in normal β cell function. ARNT expression is reduced in diabetic human islets and β cell-specific ARNT knockout mice show the impaired glucose tolerance and abnormal insulin secretion that are characteristic of type 2 diabetes.

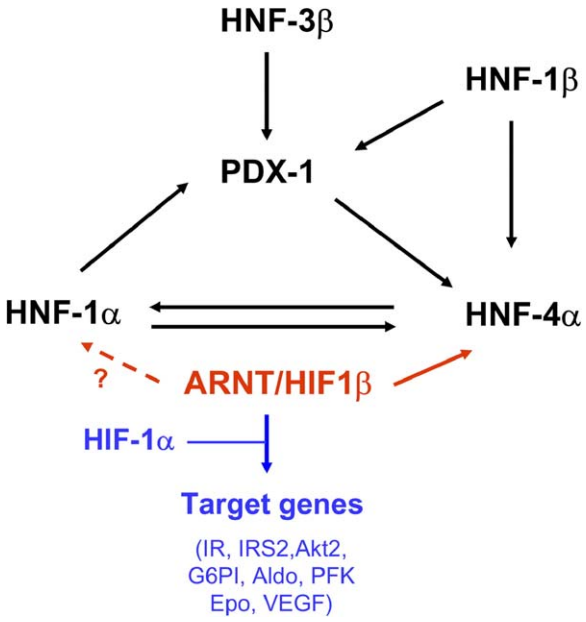

Figure 1. Model of pancreatic β cell transcriptional network

This diagram shows the transcriptional network of proteins that are essential for normal β cell function. The MODY-associated transcription factors, hepatocyte nuclear factor (HNF) 4α, HNF-1α, HNF-1β, and pancreatic homeodomain transcription factor (PDX-1), function in the nucleus of the β cell and regulate the expression of genes critical to β cell function, including the insulin gene and genes involved in the transport and metabolism of glucose. Gunton et al. (2005) demonstrate that the transcription factor ARNT/HIF1β (hydrocarbon nuclear receptor translocator/hypoxia-inducible factor 1 β) is also important for normal β cell function. Experimental reductions in ARNT expression resulted in impaired glucose-stimulated insulin secretion and reduced expression of a number of important β cell genes including HNF-4α, insulin receptor (IR), insulin receptor substrate-2 (IRS2), Akt2, glucose-6-phospho-isomerase (G6PI), aldolase (Aldo), and phosphofructokinase (PFK). Members of the hypoxia-inducible transcription factor family, such as ARNT and hypoxia-inducible factor 1α (HIF-1α), play important roles in the cellular response to hypoxia and regulate the expression of genes such as erythropoietin (Epo) and vascular endothelial growth factor (VEGF) in other tissues. This figure was adapted in part from a publication by Shih and Stoffel (2001).

Type 2 diabetes results from an inability of the pancreatic β cell to secrete sufficient insulin to adequately compensate for the prevailing degree of insulin resistance. Defining the nature of the β cell defects present in type 2 diabetes has been difficult, in part because human pancreatic β cells are inaccessible for direct study. Pancreatic islets from diabetic animal models show abnormal expression of genes involved in a multiplicity of β cell functions, suggesting a key role for transcriptional regulation of pancreatic β cell gene expression. Support for this idea comes from studies of maturity-onset diabetes of the young (MODY), an autosomal dominant form of diabetes with abnormal insulin secretion. Five of the six forms of MODY characterized to date are due to mutations in transcription factors that regulate the expression of genes key to various aspects of β cell function (Fajans et al., 2001), specifically HNF1α, HNF4α, HNF1β, pancreatic duodenum homeobox (PDX)-1, and NEUROD1/Beta2. The remaining MODY gene encodes glucokinase, the glycolytic enzyme that controls the first rate-limiting step in β cell glucose metabolism. Humans with mutations in the five transcription factors that cause MODY demonstrate reduced glucose-induced insulin secretion. Animal models in which the expression of these transcription factors have been perturbed suggest that these factors regulate the expression of the insulin and other genes that determine key β cell functions including glucose uptake and metabolism, depolarization of the β cell membrane in response to glucose, and growth and survival of the β cell (Dukes et al., 1998; Gupta et al., 2005; Johnson et al., 2003). It has been proposed that gene expression in the β cell is controlled by a complex network of transcription factors, including those responsible for the development of MODY (Shih and Stoffel, 2001).

In a study just published in *Cell* (Gunton et al., 2005), the authors suggest that another transcription factor, aryl hydrocarbon nuclear receptor translocator (ARNT) (also known as hypoxia-inducible factor 1 β or HIF1β), not previously known to be active in the β cell, plays a key role in this tissue and could potentially mediate β cell dysfunction in type 2 diabetes. Pancreatic islets were obtained from five patients with type 2 diabetes and from nondiabetic controls, and β cell gene expression was measured by oligonucleotide microarray and by real-time PCR. This screen detected reduced expression of multiple genes involved in β cell function, including certain of the MODY genes, genes involved in the uptake and metabolism of glucose, and genes within the β cell insulin signaling pathway. The most significant difference that was observed, however, was in ARNT, which demonstrated a reduction in expression of approximately 90%. To define the functional consequences of these changes, the investigators used siRNA to reduce ARNT expression in the insulin-secreting insulinoma cell line Min6. This led to impaired glucose-induced insulin secretion and recapitulated many of the reductions in gene expression seen in the islets from diabetic humans, with affected genes including HNF4α, G6PI, PFK, insulin receptor, IRS-2, and Akt2. β cell-specific ARNT knockout mice (β-ARNT) were produced in order to characterize the in vivo consequences of reduced ARNT expression. The β-ARNT mice exhibited abnormal glucose tolerance, impaired insulin secretion, and a pattern of reduced β cell gene expression that was similar to that seen in human diabetic islets and Min6 cells lacking ARNT expression.

What is known about ARNT? How might expression of this transcription factor be related to normal β cell function, and could alterations in its expression play a role in β cell dysfunction in diabetes? ARNT (HIF-1) is a member of the basic helix-loop-helix Per/AhR/ARNT/Sim (PAS) family of transcription factors and is necessary for normal embryonic development. ARNT expression and transcriptional activity increase in response to hypoxia, and ARNT's known targets include erythropoietin, glucose transporters, glycolytic enzymes, and vascular endothelial growth factor (Semenza, 1999). Its role in cellular and systemic responses to hypoxia and tumor progression has been extensively studied, and it appears to play a critical role in the maintenance

of respiratory and metabolic homeostasis. Interestingly, the mouse knockout of the aryl hydrocarbon receptor (AhR), a member of the PAS family and dimerization partner to ARNT, was found to have impaired glucose tolerance with aging (Thackaberry et al., 2003). In addition, ARNT has been reported to physically interact with HNF4α, the transcription factor that is mutated in type 1 MODY (Tsuchiya et al., 2002). Furthermore, the Per-Arnt-Sim (PAS) kinase pathway has been shown to stimulate preproinsulin and PDX-1 gene expression in rodent islets (da Silva Xavier et al., 2004).

Thus, Gunton et al. (2005) have provided convincing evidence that a reduction in ARNT expression has important negative consequences for β cell function, including impaired glucose-induced insulin secretion and reduced expression of a number of important β cell genes including HNF-4α, G6PI, PFK, insulin receptor, IRS-2, and Akt2. It therefore appears appropriate to add ARNT as a new player in the network of transcription factors that regulate normal β cell function (Figure 1). Based on the data presented, ARNT may be viewed as exerting its effects upstream of HNF4α, since levels of HNF4α were consistently reduced when ARNT expression was reduced. How ARNT expression relates to other members of this transcriptional network awaits additional studies that directly identify its downstream targets.

Do the presented data allow us to ascribe an important causal role for ARNT in the pathogenesis of type 2 diabetes in humans? Evidence for linkage between genetic variation in the ARNT gene and susceptibility to diabetes would make for a convincing argument. Demonstrating that ARNT expression is influenced by one or more of the metabolic changes that characterize diabetes or glucose intolerance, such as hyperglycemia or increased levels of free fatty acids, would also more directly connect altered ARNT expression and diabetes. Alternatively, the present findings could be the first clue to identifying an entirely novel mechanism whereby environmental stimuli may lead to β cell dysfunction and diabetes. Whatever the final outcome of these studies, this work has defined a novel role for ARNT in the transcriptional regulation of pancreatic β cell gene expression, has provided important new information about diabetic human islets, and has suggested ARNT as a potential player in the pathogenesis of the β cell dysfunction in type 2 diabetes.

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