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PPARS AS METABOLIC INTEGRATORS IN PANCREATIC BETA-CELL. NEW CHALLENGES FOR THE THERAPY OF TYPE 2 DIABETES. A MINIREVIEW

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Over the last century, changes in human behaviour and the modern lifestyle – high caloric diets and decreased physical activity - have resulted in a dramatic increase in the incidence of diabetes mellitus worldwide. In the year 2000, there were 171 million people with diabetes in the world and their number is supposed to increase to 366 million by 2030 (Wild S. *et al.,* 2004). This chronic disease is associated with diminished quality of life and reduced life expectancy rather due to its complications than to itself: specific related microvascular and macrovascular complications, including retinopathy, nephropathy and coronary artery disease. If type 1 diabetes (T1D) accounts for only 5-10% of people with diabetes being related to autoimmune mechanisms, type 2 diabetes (T2D) comprises 90% of people with diabetes around the world, in both developed and developing nations and is strongly attributable to overweight and obesity, both in adults and children (Parvez H *et al.,* 2007; Fagot – Gampagna A. and Narayan K., 2001). T2D is characterised by decreased peripheral glucose uptake and increased hepatic glucose production as a consequenc e of both reduced insulin sensitivity in the periphery and impaired insulin secretion by pancreatic β-cells. However, certain obese or overweight people don't become diabetic, because of the tremendous capacity of compensation and adaptation of pancreatic β-cells when insulin-resistance is installed. T2D occurs in such individuals when β-cells fail to compensate for the increased metabolic demands and it has been suggested that this predisposition to failure is due to a basic defect that makes islets "susceptible" or, contrary, "robust" to meet the demands for insulin of the peripheral tissues (Prentki M, Nolan CJ., 2001). Both metabolic signals and genetic factors contribute to the fine tuning of β-cell functions – insulin secretion and adaptive plasticity. T2D is commonly associated with chronic elevated levels of triglycerides (TG), free fatty acids (FFAs) and a general abnormal lipid metabolism, which contribute to the demise of β-cells ("lipotoxicity" condition) (Unger RH. and Zhou YT., 2001). Moreover, *in vivo* and *in vitro* studies have shown that, in presence of elevated glucose level, prolonged exposure to FAs results in TG accumulation in β-cells and impairment of insulin secretion, insulin gene expression and β-cell viability ("glucolipotoxicity" condition) (Prentki M, *et al.,* 2002). On the other hand, circulating FFAs assure the energetic fuel of β-cells and are essential for an efficient glucose stimulation of insulin release after prolonged fasting, both in rodents and humans (Nolan C.J. *et al.,* 2006). The understanding of the molecular machineries used by b-cells to adapt rapidly to a changing nutrient environment and to achieve correctly its functions is necessary for the development of new therapeutic strategies for the treatment and / or prevention of T2D.

Nuclear receptors (NRs) are transcription factors that regulate multiple metabolic pathways in a variety of tissues, including the endocrine pancreas. In concert with cell specific multiprotein transcriptional complexes, the *coregulators,* they regulate gene transcription by interaction with specific consensus core half-site sequences HREs (hormone responsive elements) located in the promoter of their target genes. Thus, NRs may control the expression and activity of key metabolic transport proteins and enzymes. A survey on NRs expression in adult mouse endocrine pancreas, commonly used mouse cell lines (resembling β-cells and α-cells) and human islets revealed that 18 of these transcription factors are abundantly expressed in human islets, 9 are also abundantly expressed in mouse islets and 8 others are only relatively present in mouse islets (Chuang J-C. *et al.,* 2008). Accumulating evidences support a key role played by several NRs, including the PPARs, in the physiopathology of β-cell.

The family of Peroxisome Proliferator-activated Receptors (PPARs) comprises three ligand-activated transcription factors – PPARα, PPARβ/δ and PPARγ – which have emerged as therapeutic targets in metabolic syndrome, including T2D, dyslipidaemia and inflammation. All three isotypes behave as fatty

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acid sensors and transducers of nutritional stimuli into changes in gene expression.

PPARα controls many genes of fat metabolism, in particular those implicated in mitochondrial and peroxisomal β-oxidation and fatty acid synthesis and transport. PPARα is activated by dietary mono- and polyunsaturated fatty acids and by synthetic agonists such as the fibrates (Forman BM *et al.,* 1997). PPARα is expressed mainly in the liver, kidney and skeletal muscle and is involved in fatty acid oxidation and glucose homeostasis. It is also expressed in vascular cells such as the endothelial cells, vascular smooth muscle cells and macrophages, where it exerts anti-inflammatory and anti-oxidant effects (Lefebvre P. *et al.,* 2006). First evidence that PPARα has a direct role in pancreatic β-cells came from the observation that PPARα gene expression and its target genes (UCP-2 and ACO) are down-regulated by glucose in rat islets and INS832/13 β-cells (Roduit R *et al.,* 2000). PPARα acts to protect β-cells from palmitate lipotoxicity by enhancing the expression levels of genes involved in mitochondrial and peroxisomal β-oxidation (CPT1) and lipid metabolism (GPAT, SCD1, SCD2) (Hellemans K *et al.,* 2007). In addition, its overexpression in rat β-cells restored glucose oxidation rate by the upregulation of the anaplerotic enzyme pyruvate kinase (Frigerio F *et al.,* 2010). When administered *in vivo*, PPARα–RXR ligands induced the expression of β-oxidation enzymes and stimulate palmitate oxidation in isolated islets (Zhou YT *et al.,* 1998). In insulin-resistant rodent models, PPARα agonists improve beta-cell function (Koh EH *et al*., 2003) and consistently with this, PPARα - deficiency combined with leptin – deficiency (PPARα-/-, ob/ob mice) induces pancreatic β-cell dysfunction characterized by reduced β-cells mass and decreased insulin secretion in response to glucose (Lalloyer F *et al.,* 2006). A PPARα – responsive element (PPRE) has been identified in the promoter of the PDX-1 gene, the master regulator the (pro)insulin gene transcription (Sun Y *et al.,* 2008). In human islets, PPARα agonists have been reported to prevent fatty acid-induced beta-cell dysfunction and apoptosis (Lalloyer F *et al.,* 2006).

PPARγ is an important regulator of lipid and glucose homeostasis and of cellular differentiation, predominantly expressed in adipose tissue but also present in the islets [18]. Thiazolidinediones (TZDs), the synthetic agonists of PPARγ, have been developed to improve glucose tolerance, by enhancing insulin sensitivity and indirectly restoring the function of β-cells in diabetic subjects (Nolan JJ *et al.,* 1994).

However, a direct beneficial effect of TZDs on β-cell function remains controversial and seems to involve both PPARγ - dependent and -independent pathways. PPARγ – dependent effects of TZDs on the β-cell seems to diverge in two opposite directions. More evidences have pointed to a stimulatory effect on glucose stimulated insulin secretion (GSIS) and insulin synthesis, as observed in cultured INS-1 cells stimulated 24h with rosiglitazone (Kim HS *et al.,* 2008); this PPARγ agonist also promotes the survival of pancreatic β-cells exposed to interleukin-1β (Díaz-Delfin J. *et al.,* 2007) and protects, partially, β-cells from glucolipotoxicity (Han S.J. *et al.,* 2008) and human islets from lipotoxicity (Lupi R *et al.,* 2004). The stimulatory effect on GSIS could be explained by the direct effect of TZDs on Glut2 and GK expression, two glucose sensors necessary for GSIS, via a PPARγ-responsive element (PPRE) that was identified in their promoters (Kim HI *et al.,* 2000- Kim HI *et al.,* 2002). The protection of β-cells from glucolipotoxicity seems to be correlated with TZDs potency to decrease the stress and apoptosis induced by fatty acid overexposure in β-cells (Saitoh Y *et al.,* 2008). *In vivo* studies revealed that TZDs promote β-cell survival and the maintenance of β-cell mass. β-cell targeted deletion of PPARγ in mice led to abnormal development of islets but, intriguingly, did not influence insulin secretion upon a glucose tolerance test (Rosen ED et al., 2003). Conversely, pioglitazone treatment preserved β-cell mass in db/db mice, by acceleration of cell differentiation/proliferation and reduction of apoptosis (Kanda Y. *et al.,* 2008) and endoplasmic reticulum stress (Evans-Molina C *et al.,* 2009).

Several other studies have reported a negative role for PPARγ in the β-cell. For example, Nakamichi Y. and collaborators reported that, in conditions of receptor overexpression in MIN6 beta-cells and activation by pioglitazone, glucose-stimulated proinsulin biosynthesis and insulin release were inhibited. Equally, Ito E. and collaborators found a decreased glucose-responsiveness of rat islets overexpressing PPARγ, explained by the PPARγ-induction of the UCP2 gene (Ito E. *et al.,* 2004). However, such data were reported in conditions of receptor over-expression, most probably physiologically irrelevant and when cross-talks with other transcription factors or post-translational modifications are might occur.

PPARβ/δ is not yet a target of a commercialized drug but several synthetic ligands (GW501516, GW7042 and L165041) have been developed and are currently used in research. It is ubiquitously expressed with relatively high levels in metabolically active tissues, such as muscle, liver and adipose tissue where it exerts many therapeutic effects including increasing FA uptake and oxidation in fat and muscle with increased energy expenditure, reducing glucose output and increasing lipogenesis in the liver (Shannon M. *et al.,* 2008). These effects of PPARβ/δ rely on its capacity to control, by direct or indirect transcriptional regulation, a panel of metabolic genes responsible for lipid biogenesis, transport and catabolism, glucose metabolism and inflammation status.

Within the PPARs, PPARβ/δ is the most expressed isotype in human islets (Fig.1) and is highly expressed in rodent β-cell lines (Fig.2).

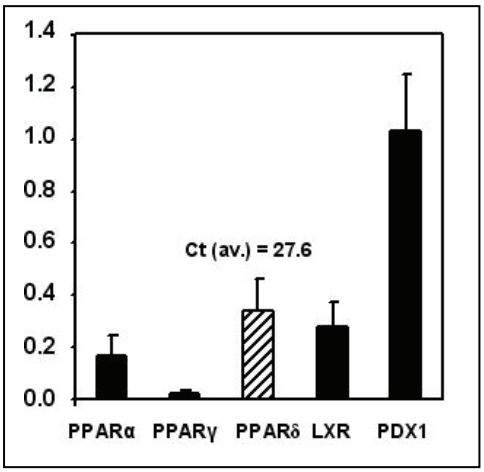

Fig. 1 Q-PCR expression of several NRs in human islets vs. PDX1 expression set at 1. (n=3, ± SD).

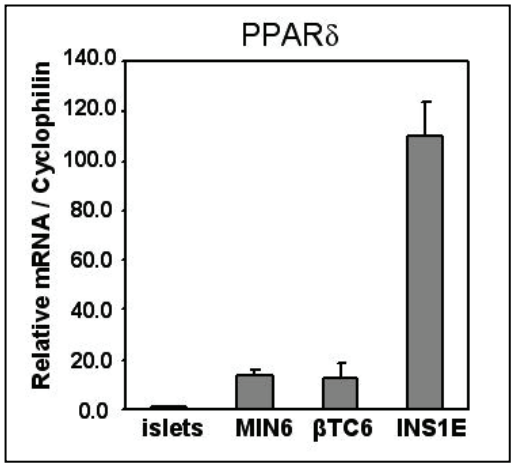

Fig. 2 Q-PCR expression of PPARδ mRNA in mouse islets and rodent β-cell lines (n=3, ± SD).

In order to identify a role of this receptor at pancreatic level, we studied the expression of several of its target genes*, in vitro* and *in vivo*. We observed that its activation with a specific ligand increased the transcription of the lipid transporter CD36 and of mitochondrial beta-oxidation enzyme CPT1 in INS1E cells (Fig. 3), thus resulting in an increased lipid uptake and enhanced FA oxidation.

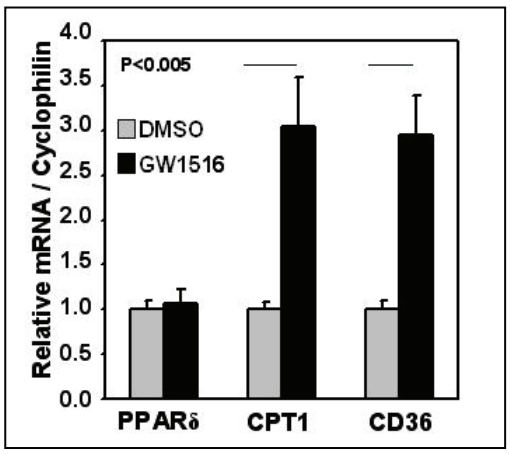

Fig. 3 The mRNA expressions of CD36 and CPT1, two target genes of PPARδ, are induced by GW501516 1μM in INS1E β-cells, after 24 hours of incubation.

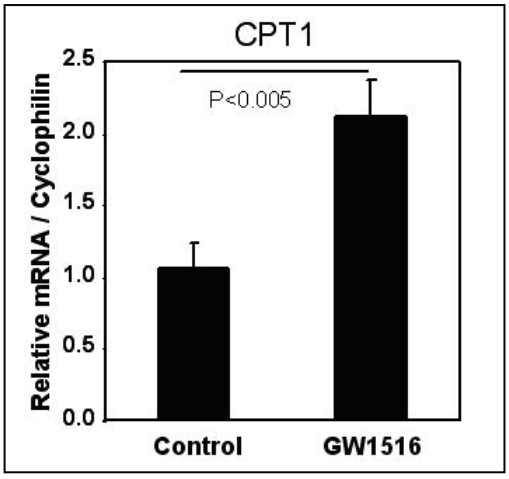

Fig. 4 The pancreatic expressions of CPT1 in C57Bl6 mice, after treatment with GW501516 for 20 days (n=7-10, ± SEM).

Similarly, treatment of C57Bl6 wild type mice with the same activator was followed by a significant increase in CPT1 mRNA level in the pancreas of these mice (Fig.4). These data indicate that PPARβ/δ behaves as a detoxification tool for β-cell when exposed to lipids. Indeed, this receptor, highly sensitive to FAs, adapts the mitochondrial capacity for their optimal oxidation, protecting against lipotoxicity-induced β-cell dysfunction (Ravnskjaer K. *et al.,* 2010). In presence of palmitate for instance, PPARβ/δ activation in a hamster β-cell line induces transcription of mitochondrial genes PGC-1α, NRF-1 and mtTFA, which contribute to enhanced mitochondrial energetic metabolism (Jiang L. *et al.,* 2010). In line with these findings, db/db mice administered orally with a selective/partial agonist of PPARβ/δ, displayed improved insulin sensitivity and islet secretory function, resulted in improved glucose metabolism (Winzell M.S. *et al.,* 2010).

CONCLUSIONS

PPAR family of NRs encompasses one of the most successful targets for drugs currently available or in development to treat multiple aspects of the metabolic syndrome. Although the preclinical studies using cellular and animal models cannot predict the risk : benefit ratio of PPAR activation in humans, they are helpful in understanding how these transcription factors act, adapt to different nutrient conditions and integrate metabolic signals in order to maintain an optimum tissue specific function. The development of SPPARM (selective PPAR modulators) molecules that activate concomitantly different PPARs with complementary roles in the management of T2D (i.e dual agonists PPARα/PPARγ) are nowadays proposed by pharmaceutical R&D as a solution for optimizing the physiological response with reduced side effects.

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