The role of oestrogen receptor beta (ERβ) in the aetiology and treatment of type 2 diabetes mellitus

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ABSTRACT

Introduction: Challenges facing the treatment of type 2 diabetes necessitate the search for agents which act via alternative pathways to provide better therapeutic outcomes. Recently, an increasing body of evidence implicates the activation of oestrogen receptors (ERα and ERβ) in the development and treatment of underlying conditions in type 2 diabetes. This article summarizes available evidence for the involvement of oestrogen receptors in insulin secretion, insulin resistance as well as glucose uptake and highlights the potential of ERβ as a therapeutic target.

Background: Recent studies indicate an association between the activation of each of the isoforms of ER and recent findings indicate that ERβ show promise as a potential target for antidiabetic drugs. *In vitro* and *in vivo* studies in receptor knock out mice indicate beneficial actions of selective agonists of ERβ receptor and underscore its therapeutic potential.

Conclusion: Studies are needed to further elucidate the exact mechanism underlying the role of ERβ activation as a therapeutic approach in the management of type 2 diabetes.

1.0 Introduction

Type 2 Diabetes (T2D) is a metabolic disease arising from failure in glucose homeostasis, caused either by impaired glucose secretion or defective insulin action. Normal insulin secretion begins with the uptake of glucose followed by a series of reactions culminating in the activation of the K_{ATP}-dependent or the CAMP-dependent pathway in pancreatic beta cells [1]. The release of insulin facilitates the uptake of glucose from the blood stream at target sites such as adipose tissue and skeletal muscles. In diabetes, this mechanism goes awry, leading to complications which affects the eye, kidney, heart and the nervous system [1-3]. Despite the heterogenic nature of the disease, it is hypothesised that insulin resistance, defined as the inability of insulin to act on peripheral tissues, is one of the major factors causing the disruption in glucose homeostasis [4]. This explains the renewed interest in developing novel therapeutic interventions which can correct defective insulin action.

2.0 Oestrogen receptors: structure and classical functions

Oestrogen plays significant roles in the development and maintenance of reproductive functions, but has also been shown to play biologically significant roles in the nervous, immune, musculoskeletal and cardiovascular systems [5]. 17β-estradiol (E2) has been reported as the most potent, endogenous agonist of oestrogen receptors (ERs) and its significant role in regulating the expression of these receptors is well established [6,7]. E2 diffuses through the cell to reach the nucleus and bind to the oestrogen receptor (ER). This complex mediates transcription by enabling the receptor to interact with oestrogen response elements (ERE) which are present in the promoter region of oestrogen-responsive genes. Thus, gene expression is regulated by recruiting activators or repressors and generating a cellular response.

 The discovery of the first oestrogen receptor, ERα, came in the 1960s when Elwood Jensen described the effects of an oestrogen binding protein [8]. Three decades later, ERα knockout mouse models revealed that ERα was not the sole mediator of oestrogen signalling. Further characterisation studies resulted in the discovery of the ERβ receptor, which was hypothesised to work as an alternative to substitute the knocked-out ERα receptor [9] and transmembrane ERs such as the G protein-coupled estrogen receptor (GPER), believed to have signalling and transcriptional regulatory roles [10]. However, it was studies involving ERα$^{-/-}$ and ERβ$^{-/-}$ and aromatase knock-out (ArKO) mouse models that helped elucidate the individual actions of ERα and ERβ receptors [11 - 13]. Aromatase gene is responsible for the endogenous production of E2 [14]. These models allowed a greater understanding of the distinct roles played by ERα and ERβ receptors *in vivo.* One of such studies investigated the differential mode of actions of the two receptors by studying their transfection into neuroblastomas. It was observed that transfected cells with activated ERα receptor had

increased number and length of neurites whereas transfected cells with activated ERβ exhibited neurite elongation [15]. Moreover, Dupont et al [16] reported that while ERα is indispensable for ovulation and interstitial glandular cell development while ERβ is involved in ovarian follicle generation. In relation to insulin secretion, studies involving the use of agonists of both ERα and ERβ in ERαKO. ERβKO or wild type mice indicated that ERα is largely involved in the upregulation of pancreatic insulin content while the role of ERβ in this regard is not yet fully understood [17].

Moreover, the use of selective agonists for each class of receptors in cell lines which presented both ERα and ERβ produced different responses. Incubation of immortalized epithelial cell lines with selective ERα agonists resulted in cell proliferation whereas the use of selective ERβ agonists resulted in cell growth inhibition [18]. Different actions of ERα and ERβ at the promoters of the genes they regulate suggests that the global response of an agonist depends on the balance between the activities of both receptors [19] and opens the possibility for exploitation of these differential actions for selective therapies in the diseases involving oestrogen receptors.

3.0 Roles of ERs in insulin secretion and glucose tolerance

An increasing body of evidence indicates the involvement of oestrogen receptors (ER) in glucose homeostasis [14, 20-22]. ERs were first linked to obesity due to their expression in the adipose tissues. Studies in oestrogen receptor knock-out models further confirm the association between oestrogen receptors activation, insulin resistance and glucose homeostasis [14, 17, 23]. The activation of ERα by 17beta-estradiol (E2) has been shown to stimulate insulin biosynthesis via a pathway involving the activation of ERK1 and ERK2 [24 - 26]. Nadal et al [26] further reported that hyperactivation of ERα by xenoestrogens (such as bisphenol A) can induce excessive insulin signalling, leading to insulin resistance. Following

a study which investigated various mechanisms through which oestrogen receptors regulate glucose metabolism, Weigt et al [24] reported that the activation of ERα elicits muscular GLUT4 expression while the activation of ERβ leads to hypertrophy in muscle fibres. The observation of obesity and insulin resistance in aromatase gene knock-out (ArKO) mice further confirmed a role for ERβ [25, 26].

While severely impaired insulin sensitivity and glucose tolerance has been established in ERα-knock out mice [7, 14, 27, 28], recent studies report a role for ERβ in glucose metabolism. For instance, ERβ isoform has been shown to be widely distributed and is the more dominant isoform found in skeletal muscle [14, 29, 30], a site for glucose uptake. While aromatase deficiency and ERα polymorphisms have been associated with insulin resistance and type 2 diabetes [27], the role of ERβ in the development of type 2 diabetes is still unfolding. High-fat fed mice treated with ERβ selective agonist showed decreased expression of GLUT4 [30], the insulin-regulated glucose transporter found in adipose tissue and skeletal muscle, as well as impaired insulin sensitivity and glucose tolerance [31]. These effects are opposite to actions reported for ERα [30].

Studies have shown that oestrogen interacts with receptors in the plasma membrane as well as in the nucleus; eliciting both non-genomic and genomic responses respectively [32]. Though most genomic actions of oestrogen are generally believed to be consequences of the interaction of oestrogen with nuclear receptors, it has been reported that changes in gene expression can also result from oestrogen signalling via membrane-bound receptors [32, 33]. Though mechanisms underlying these actions are currently poorly understood and the membrane expression of ERα and ERβ in small quantities has been reported [34], G protein coupled ERs (GPER) have been largely implicated in this regard [33]. It has been reported that signalling through GPER is dependent on epidermal growth factor receptor and ERK

activation via the actions of nonreceptor tyrosine kinases of the Src family [35, 36]. Prossnitz and Barton [36] further reported that GPER's activation by E2 leads to cAMP production, intracellular calcium recruitment and PI3K activation. These processes are critically involved in insulin secretion from beta cells and have attendant effects on glucose metabolism. This may also partly account for available evidence for the actions of oestrogens on cellular activities leading to the elevation of intracellular calcium concentration, NO production and the activation of kinases [37]. The involvement of oestrogens in these processes suggest involvement in the short-term regulation of metabolism and homeostasis.

4.0 ERβ activation, GLUT4 expression and implications for the treatment of type 2 diabetes

Recent studies have shown that the activation of the ERβ triggers the closure of the ATP-sensitive potassium channel (K_{ATP}-channel) leading to membrane depolarization, the opening of the voltage dependent calcium channel (VDCC), influx of extracellular calcium and insulin secretion in a glucose-dependent manner [5, 38]. Other studies demonstrated that E2 increases the level of cyclic GMP (cGMP) in pancreatic beta cells. This promotes the recruitment of intracellular calcium resulting in increased insulin secretion in pancreatic beta cells [39]. These actions strongly suggest a role for ERβ in the cascade of events leading to physiologic insulin secretion and the potential use of ERβ receptor agonists in the treatment of type 2 diabetes. Effects observed in *in vitro* studies were also investigated in *in vivo*. Alonso-Magdalena et al. [5] reported both acute and chronic beneficial actions of a novel ERβ selective agonist, WAY200070, on insulin secretion and glucose tolerance in mice with chemically-induced type 2 diabetes. A major conclusion of studies investigating anti-diabetic actions of ER agonists is that effects observed are largely dependent on cellular ERα:ERβ expression ratio and that further studies are needed to explain the interplay between the differential expression of these receptors vis-à-vis their role in glucose metabolism [5, 30].

Evidence for GLUT4 modulation by ERβ could provide a possible avenue for the treatment of diabetes mellitus. Investigations, aimed at evaluating the effects of E2 on GLUT 4 sub-cellular localization or expression, conducted on white adipose tissue were less successful compared to studies which analysed ER expression in mice skeletal muscle. Barros et al. [40], for instance, observed the co-localization of ERα and ERβ in the nuclei of gastrocnemius muscle in mice and argued that E2 activity is a function of the ratio at which ERα and ERβ are expressed in the tissue. The study also indicated that ERα:ERβ ratio influences GLUT 4 expression and its sub-cellular localization. In a study conducted using ArKO mice, Barros et al. [40] showed that GLUT4 was dually modulated and that treatment with an ERβ agonist, 2,3-bis(4-hydroxyphenyl)propionitrile (DPN) elicited blunted GLUT4 expression. Though no overall conclusion can be made about whether this is a general characteristic of all ERβ ligands [41, 42], this observation partly informed the current understanding that the inhibition of ERβ (e.g. by using an antagonist of ERβ expression), could result in changes to ERα:ERβ ratios in cells where they are co-expressed (such as skeletal muscle and adipose tissue [43]). These observations indicate a GLUT4-modulatory role for ERβ and suggest that the receptor could be an important therapeutic target for type 2 diabetes.

Endogenous regulation of ERβ levels in skeletal muscle by steroid hormone, androgen, plays significant roles in the modulation of muscle physiology leading to decreased ERβ expression and subsequent inhibition of insulin resistance [40]. Association between insulin resistance and diabetes mellitus in hypogonadal patients with low levels of testosterone, a hormone previously shown to down regulate ERβ activity, has also been established [44, 45]. Previous observations together with the discovery of novel agonists of ERβ (such as 4-(butyryloxy)benzoate) point towards the importance of producing tissue-specific selective ERβ modulators as alternate potential therapeutic agents for the treatment of type 2 diabetes

[46]. Tetrahydrochrysene (R,R-THC) is another promising agent which acts as a selective ERα agonist and ERβ antagonist [46].

The production of drugs which selectively target transcription factors regulating GLUT4 expression, such as nuclear factor κB response element (NF-κB-RE) and specificity protein 1 (SP1), represents another therapeutic approach in this regard [47, 48]. The activation of ERα and ERβ have been demonstrated influence the actions of SP1 resulting in differing GLUT4 expression levels. However, these actions are both gene specific and variable in nature [49, 50]. It is hypothesised that ERα works to upregulate SP1 action resulting in increased GLUT4 expression whereas ERβ works to inhibit the action of SP1. However, the exact mechanism by which this is achieved is yet to be full elucidated.

5.0 Conclusion

In conclusion, improved understanding of the regulatory effects of estradiol (E2) on the expression of GLUT4 via interactions with ERα and/or ERβ may represents a significant milestone in the search for pathways involved in glucose homeostasis and insulin resistance. Available information clearly indicates the potential beneficial effects of selective activation of the ERβ as a target for the treatment of type 2 diabetes. However, there is a need for careful study of the underlying mechanism of action and other attendant effects of ERβ receptor agonists.

6.0 Conflict of interests

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