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Role of estrogen receptors alpha, beta and GPER1/GPR30 in pancreatic beta-cells

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1. ABSTRACT

Estrogen receptors (ER) are emerging as important molecules involved in the adaptation of beta-cells to insulin resistance. The onset of type 2 diabetes is marked by insulin secretory dysfunction and decreased beta-cell mass. During pregnancy, puberty and obesity there is increased metabolic demand and insulin resistance is developed. This metabolic state increases the demand on beta-cells to augment insulin biosynthesis and release. In this respect, ERalpha is directly implicated in the E2-regulation of insulin content and secretion, while ERbeta is in the E2-potentiation of glucose-induced insulin release. Both receptors develop their actions within the physiological range of E2. In addition, the G protein-coupled estrogen receptor (GPER1/GPR30) seems to be implicated in the E2-regulation of stimulus-secretion coupling in the three cell types of the islet. The increased demand of insulin production for long time may lead to beta-cell stress and apoptosis. ERalpha, ERbeta and GPER1/GPR30 are involved in preventing beta-cell apoptosis, impeding the loss of critical beta-cell mass. Therefore, estrogen receptors may play an essential role in the adaptation of the pancreas to insulin resistant periods.

2. INTRODUCTION

Blood glucose homeostasis is essential for an appropriate function of the human and animal organisms. This process involves several different tissues, including the liver, skeletal muscle, adipose tissue, brain and endocrine pancreas (1,2). The endocrine pancreas is a key organ for blood glucose regulation; its physiological unit is the islet of Langerhans. Islets are distributed throughout the pancreas and their number reaches one million per organ in humans. Each islet contains from 1000 to 3000 cells of five different types (3-5). The most abundant cells are beta-cells, which synthesize and release insulin in response to an increase of extracellular glucose. Insulin is the only hormone in the body able to reduce blood glucose. The dysfunction in the biosynthesis and release of this hormone leads to the widespread pathology Diabetes Mellitus.

In humans, ovarian hormones influence insulin sensitivity throughout the menstrual cycle, during pregnancy, and in the menopausal transition. Estrogens as well as progestins used for contraception and hormone replacement therapy (HRT) affect glucoregulation (6). Although less known, environmental estrogens or

endocrine disruptors such as bisphenol-A have been associated with higher incidence of type-2 diabetes in adults (7).

It has been described, in human and animal models, that estrogens exert both beneficial and detrimental effects on blood glucose homeostasis, depending on their concentration, duration of exposure, gender and other variables (8-10). The conclusion that may be drawn from multiple studies on the effect of estrogens on blood glucose homeostasis is that lower than usual estrogen levels result in insulin resistance and impaired glucose tolerance. In this situation, estrogen replacement at physiological levels improves blood glucose homeostasis. However, when an excess of estrogen is present, because of HRT or oral contraception, both glucose intolerance and insulin resistance develop (8). When the direct effects of estrogen on the beta-cell function and viability are reviewed, a clear protective effect is observed against external insult (8,11,12). Regarding insulin secretion, both a decrease and an increase in insulin secretion are obtained, depending on the estrogen dose (13-15). Notably, if these actions are induced by an endocrine disruptor when there is no need for estrogenic signaling, it may induce insulin resistance (9,16).

Pancreatic beta-cells adapt to peripheral insulin resistance by increasing insulin biosynthesis, secretory response to nutrients and cell mass. Estrogen receptors are emerging as important molecules for glucose homeostasis (17,18) and are involved in the adaptation of the pancreatic beta-cell function to insulin resistance during those periods where estrogen levels change, such as puberty, pregnancy and menopause (19). In this review we will describe the state of research about the physiological role that estrogen receptors ERalpha and ERbeta have in the beta-cell function and discuss whether the G protein-coupled estrogen receptor (GPER1), also named GPR30, also plays a role in the function of this particular cell type.

3. GLUCOSE-INDUCED INSULIN SECRETION/INSULIN BIOSYNTHESIS COUPLING IN beta-CELLS

For an appropriate functioning of beta-cells and therefore an adequate control of glucose homeostasis, insulin release and insulin biosynthesis must be well connected (20,21). Pancreatic beta-cells are electrically excitable (22), their electrical activity consists of oscillations in the membrane potential from electrically silent periods to depolarized plateaus on which Ca^{2+}-action potential originates. In the generation of this particular electrical activity, two ion channels are crucial, ATP-sensitive K^+ channels (K_{ATP} channels) and voltage-gated L-type Ca^{2+} channels. K_{ATP} channels control the resting membrane potential and determine the electrical resistance of beta-cells. When K_{ATP} channels remain open, membrane resistance is low and then small currents only minimally affect plasma membrane potential and, consequently, insulin release. However, when K_{ATP} channels are mostly closed, the membrane resistance is high and then, the small currents greatly affect plasma membrane potential and thus insulin release. This is the case of many insulin secretion regulators, including E2, as will be discussed later.

The classical stimulus-secretion coupling that induces insulin release involves the closure of K_{ATP} channels by the increase of the intracellular ATP/ADP ratio (23) and diadenosine polyphosphates (DPs) (24) because of glucose metabolism. The channel closure induces membrane depolarization that activates voltage-operated Ca^{2+} channels and Ca^{2+} influx (25). The set of channels of the beta-cell plasma membrane generates the oscillatory electrical activity mentioned above. As a consequence, an intracellular calcium concentration ($[Ca^{2+}]_i$) oscillatory pattern is originated (26-28) triggering a pulsatile insulin secretion (29,30). All current models of stimulus-secretion coupling in this cell type emphasize the essential role that K_{ATP} channels play in the transduction of glucose metabolism into electrical activity and $[Ca^{2+}]_i$ signals. The latter is the key for insulin release, since every physiological or pharmacological agent that increases $[Ca^{2+}]_i$ either dependently or independently of the closure of K_{ATP} channels induces insulin secretion (31).

In addition to glucose-induced insulin release, a dynamic regulation of insulin biosynthesis is needed for an appropriate control of blood glucose by insulin. When insulin stores are partially emptied because of insulin release, they are rapidly refilled by the activation of insulin biosynthesis. Therefore, insulin stores are continuously replenished, providing an appropriate release of the hormone and maintaining blood glucose within the physiological range. The regulation of insulin biosynthesis by glucose includes both translational (32) and transcriptional (20,33) mechanisms.

The role that estrogen receptors play in E2 regulation of glucose-induced insulin secretion and insulin biosynthesis is described in this review.

4. ERalpha, ERbeta AND GPER1/GPR30 ARE EXPRESSED IN beta-CELLS

Classically, estrogens exert their effects by interacting with two known estrogen receptors, ERalpha and ERbeta, that act as transcription factors to regulate gene transcription (34,35). It is now accepted that both ERalpha and ERbeta also act extranuclearly to initiate signaling cascades that regulate multiple cell functions (36-39). In 2005, two groups independently proposed that the orphan G protein-coupled receptor GPR30 binds E2 and rapidly activates different signaling pathways (40-42). Although the task of GPR30 as an estrogen receptor is still controversial (43,44), some physiological roles are emerging (45-47). Now GPR30 has been named GPER1 and classified as a membrane estrogen receptor by the International Union of Pharmacology.

Inmunofluorescent staining using a battery of antibodies as well as qualitative and quantitative RT-PCR experiments demonstrated that both ERalpha and ERbeta are expressed in mouse beta-cells (12,48,49). Western blot studies established the existence of two ERalpha isoforms,

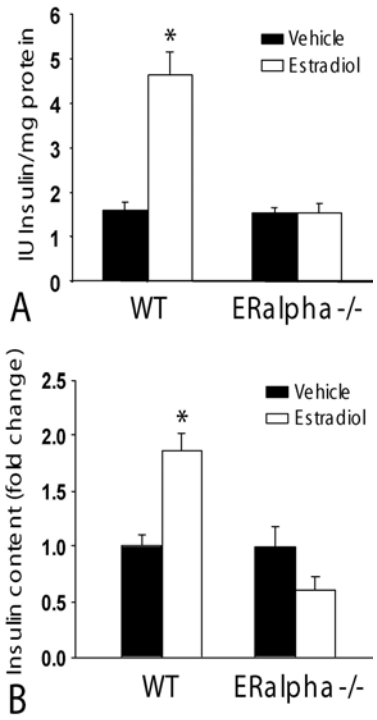

Figure 1. E2 effect on insulin content in ERalpha-/- mice. A) Insulin content obtained in cultured islets from wild-type and ERalpha-/- mice exposed to either vehicle or 1 nM E2. *p<0.001 compared to vehicle. B) Insulin content in islets obtained from animals treated with 100 µg/kg/day E2 (white columns) or vehicle (black columns) for 4 days. E2 treatment in ERalpha-/- mice has no effect. *p<0.01. Data are reproduced from reference 48.

the long 66-KDa and a shorter 58 KDa isoform in mouse islets (50,51). In human islets, a novel 52-kDa ERalpha isoform was detected in the absence of the classic 67-kDa protein (50). Immunofluorescence experiments demonstrated the expression of both ERalpha and ERbeta in human islets (12). In the clonal beta-cell line MIN6, the 66-KDa isoform of ERalpha was expressed and immunocytochemistry experiments showed the presence of ERbeta (12,51). The location of both receptors is important for their function (52). In mouse and human beta-cells, they are localized mainly in the cytoplasm, yet, they are also found in the nucleus (12,48,49).

A decade ago, a non-classical membrane estrogen receptor (ncmER) different from ERalpha and ERbeta was proposed in pancreatic beta-cells and in glucagon-releasing alpha-cells (49,53-55). Recently, the expression of GPER1/GPR30 in alpha, beta and delta cells has been demonstrated (12,13,56). Its mimetism of some of the actions previously attributed to the ncmER, suggest that GPER1/GPR30 and ncmER may be the same receptor.

5. THE ROLES OF ERalpha

The main function of the pancreatic beta-cell is the biosynthesis and release of insulin in response to external stimuli. These two parameters, together with the regulation of beta-cell mass are the keys for the endocrine pancreas to adapt to peripheral insulin resistance. During insulin resistant periods such as pregnancy and puberty and when there are certain metabolic disorders, estrogen signaling is important for the pancreas to adapt to the new metabolic situation (12,19). It was known for a long time that E2 increased pancreatic insulin content and secretion in rodents (57,58, reviewed in 8). Recent experiments indicate that *in vivo* treatment of male mice with E2 100 µg/kg/day during 4 days increases insulin content and insulin secretion in response to high glucose. This action is abolished by treatment with the pure antiestrogen ICI182780, pointing to a classical ER mediated effect (16). However, E2-treated mice present insulin resistance and therefore the increase in insulin content and release could be due to the adaptation of the endocrine pancreas to peripheral insulin resistance or to a direct action on beta-cells. Our analysis of the action of E2 exposure on islets in primary culture during 48 hours has shown results indicating that E2 increases pancreatic insulin content as a consequence of an up-regulation of insulin mRNA levels, while no change in beta-cell viability or beta-cell division has been identified (48). Taking insulin content as an end-point, we have described that its up-regulation is elicited by the ERalpha agonist PPT but not by the ERbeta agonist DPN. When genetically modified mice were used E2 increased insulin content in cultured islets from wild-type (WT) but not in islets from ERalpha-/- mice (Figure 1A). The absence of an effect on insulin content up-regulation in ERalpha-/- mice was observed *in vivo* in mice treated with 100µg/kg/day during 4 days (Figure 1B) (48). These ERalpha–mediated actions involved Src and ERK1/2 kinases and did not seem to require the binding of ERalpha to an estrogen response element (ERE) in the DNA given that the insulin gene does not have an ERE. Moreover, E2 and the endocrine disruptor, bisphenol-A are equally effective, while it is well known that the binding of bisphenol-A to ERalpha has a low transcriptional activity via ERE binding (48,59). In summary, E2 increases insulin gene expression, insulin content and insulin release via an ERalpha/ERK1/2 pathway. These actions may be important for the adaptation of beta-cells to the higher demand of insulin during pregnancy and other metabolic altered states (reviewed in 19).

Another relevant action of E2 on mouse beta-cells is its protective effect against apoptosis in a model of diabetes induced by streptozotocin (STZ) (51,60) as well as in isolated human islets in response to proinflammatory cytokines-induced cell death (11). On one hand, human islets incubated for 4 days in the presence of 10µM estradiol developed resistance against cytokines-induced apoptosis. The involvement of ERalpha and ERbeta is suggested by the partial reversal of this E2 protection by ICI182,780 (11). On the other hand, Le May *et al.* (51) showed that female mice were protected against STZ-induced beta-cell loss compared to male ones that were not. In addition, estradiol protects male mice from STZ-induced beta-cell loss, apoptosis and STZ-induced diabetes. The involvement of ERalpha in the female protection is suggested when ERalphaKO female mice lose their protection against beta-cell loss induced by STZ (51).

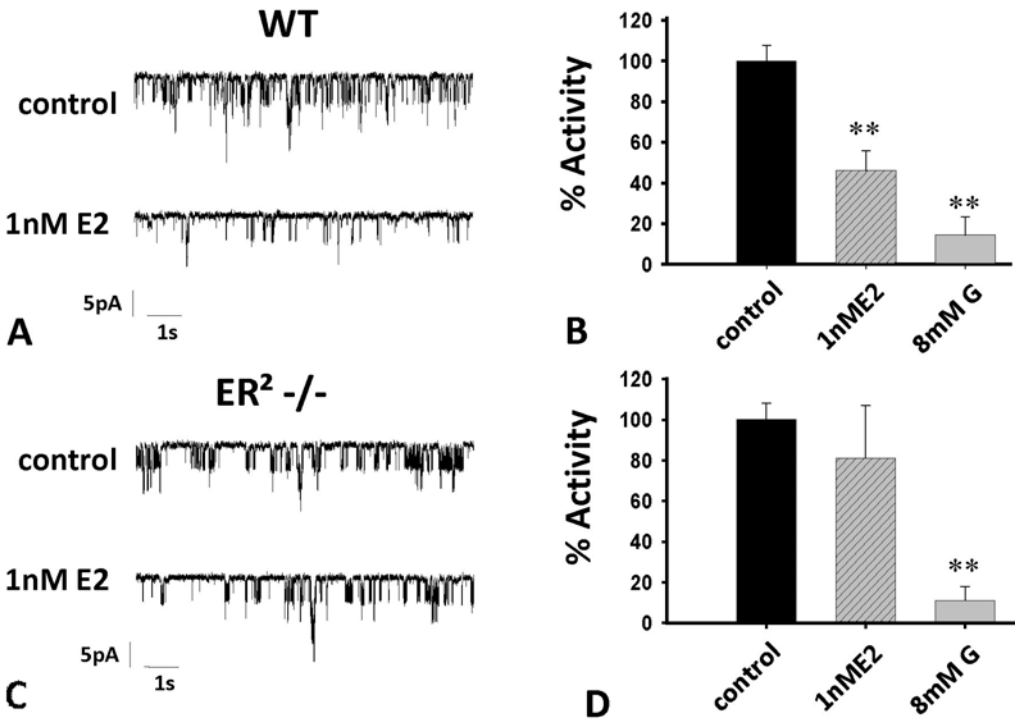

Figure 2. E2 regulation of K_{ATP} channel activity is abolished in beta-cells from ERbeta-/- mice. A) E2 at 1nM decreased K_{ATP} channel activity in intact isolated beta-cells from WT mice. The records show the K_{ATP} channel activity before applying E2 and 7 minutes after applying E2. B) Percentage of activity of the K_{ATP} channels elicited by vehicle, 1nM E2 and 8mM glucose. C) E2 1nM had no significant effect on K_{ATP} channel activity in isolated beta-cells from ERbeta-/- mice. As in A, the records show K_{ATP} channel activity before applying E2 and 7 minutes after applying E2. D) Percentage of activity of the K_{ATP} channels elicited by vehicle (control), 1nM E2 and 8mM glucose. **p<0.01 Student's t-test comparing 8mM G with control. Modified from reference 64 with permission of The Endocrine Society.

Recently, the same group has used an ERalpha knock-in mouse with a mutation in the DNA-binding domain to elegantly demonstrate that the action of ERalpha is not by binding to ERE, because this mechanism is impeded in these knock-in mice (12,61). In addition, they suggest that ERbeta may also be involved in the protective effect of E2, since the incidence of STZ-induced diabetes in betaERKO is increased compared to WT. E2 still partially protect alphabetaERKO against H_2O_2-induced apoptosis, which suggests the involvement of yet another receptor in this beneficial effect of E2 on beta-cells. Therefore, although the role of ERalpha is prominent in apoptosis prevention, ERbeta and even GPER1/GPR30, may be involved in this process as well (62).

6. THE ROLES OF ERbeta

Physiological concentrations of E2 (100pM-10nM) rapidly release insulin in synergy with glucose, both *ex vivo* in isolated islets and *in vivo* (15,16). When the E2 regulation of stimulus-secretion coupling was studied, it was found that K_{ATP} channels close in few minutes (4-7 minutes for maximal effect). The closure of K_{ATP} channels coincided with the potentiation of glucose-induced electrical activity, $[Ca^{2+}]_i$ oscillations and insulin secretion (15). E2-induced K_{ATP} closure was a PKG-dependent process involving the rapid increase of cGMP (63). Recent data indicated that the Atrial Natriuretic Peptide Receptor (also named Guanylate Cyclase-A receptor) participated in this process (64). E2 conjugated to albumin (E2-BSA) or horseradish peroxidase (E2-HRP) mimicked the effect of E2 on calcium signaling. This indicated that the effect was triggered at the plasma membrane (15,49,55). Later studies have revealed that the pure antiestrogen ICI182,780 did not block neither the E2-regulation of glucose-induced Ca^{2+} signals (55) nor insulin release *ex vivo* (65) or *in vivo* (16). These, together with the different pharmacological profiles presented by the membrane estradiol binding site (49,55) led us to assume that the rapid insulinotropic effect induced by E2 was exclusively due to a non-classical membrane estrogen receptor (ncmER). Although the existence of this receptor is not denied (see Roles of GPER1/GPR30 Section), the situation is more complicated than we initially thought. Recent evidence obtained with ERalpha-/- and ERbeta-/- mice indicate that ERbeta is involved in the E2-induced blockade of K_{ATP} channels (64) (Figure 2). Moreover, activation of ERbeta by the agonist DPN reduced K_{ATP} channel activity in beta-cells from WT but not in cells for ERbeta-/- mice. Moreover, DPN increased glucose-induced $[Ca^{2+}]_i$ signals and insulin release, pointing to the fact that the direct activation of ERbeta imitates E2 action (64) (Figure 3A,B). In summary,

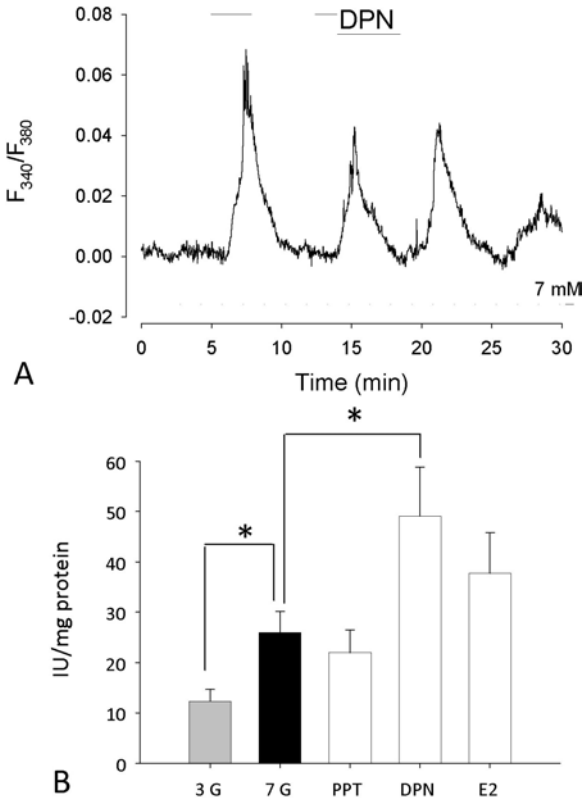

Figure 3. Activation of ERbeta by DPN enhances glucose-induced $[Ca^{2+}]_i$ signals and insulin secretion. A) Typical $[Ca^{2+}]_i$ response of an islet of Langerhans in the presence of 7mM glucose to 1nM DPN applied for the period indicated by the bar. The Ca^{2+}-dependent fluorescence of Fura-2 is expressed as the ratio F_{340}/F_{380}. Note that $[Ca^{2+}]_i$ oscillations are generated on a non-oscillatory plateau produced by 7mM glucose. B) Glucose-induced insulin secretion from islets exposed to 3mM glucose (3G), 7mM glucose (7G), 7mM glucose and 1nM PPT (PPT), 7mM glucose and 1nM DPN (DPN) or 7mM glucose and 1nM E2 (E2) for 1 hour. * $p<0.05$ Student's t-test. Modified from reference 64, with permission of The Endocrine Society.

physiological E2 concentrations (1nM) elicited the closure of K_{ATP} channels and potentiated glucose-induced insulin release in an ERbeta dependent manner.

Although, the main function of ERbeta in beta-cells is the rapid regulation of K_{ATP} channels and insulin secretion, compared to ERalpha, a secondary role in cytoprotection has been suggested (12). Female betaERKO mice are mildly predisposed to STZ-induced diabetes and present higher fed blood glucose levels than WT. Although DPN provided only minor islet protection, the specific ERbeta antagonist THC impaired E2 protection from H_2O_2-induced apoptosis (12).

Other evidence of the involvement of ERbeta in blood glucose homeostasis is based on data showing that betaERKO present improved insulin resistance and higher glucose tolerance after a high fat diet compared to WT. The contribution that beta-cells may have on this protective effect in the absence of ERbeta has not been studied (66).

7. THE ROLES OF GPER1/GPR30

As previously mentioned, a decade ago, our group described the existence of a ncmER in pancreatic beta and alpha-cells. In the previous sections we have described how ERbeta is responsible for many of the actions previously attributed to the ncmER. However, the existence of a membrane E2 binding site with a different pharmacological profile than that of ERalpha and ERbeta, in both beta and alpha-cells was clear (49,53,55). Sensitivity to the Pertussis toxin, indicating that the ncmER was a G protein-coupled receptor, was clearly obtained in alpha-cells (53).

New results suggest that GPER1/GPR30 may be the ncmER that we previously proposed. In view of the up-to-date data, its role as an estrogen receptor at supraphysiological doses of the hormone (100nM-10μM) is plausible, yet, its role in response to physiological doses (10pM-10nM) is still unclear.

Recently, Mårtensson *et al* 2009 (56) demonstrated the expression and participation of GPER1/GPR30 in the insulinotropic effect of E2. These authors showed that islets from GPR30+/+ respond to a pharmacological dose of E2 (5μM) with an increase of insulin secretion under both low (1mM) and high (20mM) amounts of glucose. E2 action was completely abolished in islets from GPR30-/- mice. Low glucose-induced glucagon secretion was negatively regulated by E2 in GPR30+/+ islets but not in GPR30-/-. These results strongly suggest that GPER1/GPR30 mediates the supraphysiological stimulation of insulin release and the inhibition of glucagon secretion in pancreatic islets. This work demonstrated that GPER1/GPR30 was involved in E2 signaling (56). However, the role of GPER1/GPR30 acting as a genuine E2 receptor has been challenged by some recent reports (43,67). This question has been addressed in a recent paper by Balhizen *et al*, 2010 (13) by using G-1, a newly described selective agonist for GPER1/GPR30 (68). The authors used islets from NMRI mice to demonstrate that both E2 and G-1 stimulate insulin secretion in the presence of high glucose concentrations (12mM). Remarkably, no effect was obtained from either E2 or G-1 on insulin release in the presence of low glucose (1mM) contrary to what had been previously reported (13,56). In addition, they described an inhibitory action on low glucose (1mM)-induced glucagon release but no effect on glucagon release with high glucose (12mM). Interestingly, GPER1/GPR30 is expressed in somatostatin-containing delta-cells and both G-1 and E2 decreased glucose-stimulated somatostatin release (13).

Notably, there are marked differences in the efficacy of G-1 and E2 in the three different cell types. The effect of E2 was one order of magnitude higher compared to that of G-1 on glucose (12mM)-induced insulin release

yet it was equally effective on glucagon and somatostatin release. Moreover, ligand receptor binding studies pointed that G-1 has a stronger affinity for GPER1/GPR30 than E2 (69). There are different explanations for this discrepancy (13), yet one of them may be that ERbeta plays an important role in beta-cell stimulus-secretion coupling (64 and previous section in this review). In addition, the role of ERbeta on glucagon and somatostatin secretion is still unknown.

It is noteworthy that no molecular approach has been used to directly demonstrate that G-1 is acting through GPER1/GPR30, i.e.: islets from GPR30-/- mice. We must keep in mind that the selectivity of newly developed drugs is usually challenged with time and therefore, the use of genetically modified mice or siRNA is highly recommended. One example of this is the binding of G-1 to the variant ERalpha-36 and the induction of nongenomic signaling (67).

In addition to the involvement of GPER1/GPR30 in the E2-regulation of stimulus-secretion coupling in the different cells types of the islet, it has been recently described that GPER1/GPR30-/- mice present higher STZ-induced diabetes than WT. In addition, G-1 protects islets cells against H_2O_2- or cytokine-induced apoptosis (12). It is important to note that cytoprotection occurs independently of nuclear events (61).

In relation with the pathways triggered by E2, both GC-A and Adenylate Cyclase (AC) seem to play roles depending on the receptor activated by E2. Physiological doses of E2 (1nM) were shown to rapidly increase cGMP levels in islets of Langerhans in the presence of a stimulating glucose concentration (8mM) yet cAMP levels were unchanged (63). Moreover, the E2-induced decrease of the activity of K_{ATP} channels was prevented using a PKG inhibitor but unaffected in the presence of a PKA inhibitor (63). Experiments using GC-A KO and ERbeta-/- islets indicated that the ANP receptor and ERbeta play a key role in E2-enhanced cGMP levels (64). As described in other tissues (42,70) GPER1/GPR30 stimulates the generation of cAMP in islets based on the effect of G-1 (13). E2 generated cAMP as well but in response to supraphysiological concentrations of 100nM and 5μM (13).

Our group showed that the inhibitory effect of E2 on glucagon secretion is prevented by the pertussis toxin, which suggests the involvement of $Galpha_{i/o}$ (53). This G-protein subfamily inhibits adenylyl cyclase and therefore cAMP production, which in turn blocks glucagon secretion (71). However, although GPR30 activation with G-1 also decreases glucagon secretion, similarly to E2, it also increases cAMP content in islets. This increase in cAMP is opposite to what would be expected under the hypothesis that GPR30 mediates glucagon secretion inhibition. Therefore, it remains to elucidate specifically the effect of GPR30 activation on cAMP levels in pancreatic alpha-cells to support the hypothesis that GPR30 is the ncmER in alpha-cells.

8. ENDOCRINE DISRUPTOR ACTIONS

In addition to the physiological role that E2 binding to estrogen receptors has in the islet of Langerhans, environmental estrogens acting as endocrine disruptors bind to these receptors and exert rapid responses in different cell types (72-74).

In pancreatic beta-cells, low doses (1nM) of the endocrine disruptors diesthylestilbestrol (DES) and bisphenol-A (BPA) potentiated glucose-induced Ca^{2+} signals and activated the transcription factor CREB (49,75). *In vivo* experiments showed that BPA rapidly increased plasma insulin and decreased blood glucose (16). In glucagon containing alpha-cells, both DES and BPA rapidly reduced low glucose-induced Ca^{2+} signals (53). In all these experiments the antiestrogen ICI182,780 had no effect, indicating that a ncmER may be involved (54). Now, it is unclear whether these actions are through a ncmER (perhaps GPER1) or via extranuclear ERβ, that might be insensitive to the antiestrogen, or to both receptors.

In addition to these rapid actions, BPA exerted a long term regulation of pancreatic insulin content, demonstrated both *in vivo* (16) and *in vitro* (48). These *in vitro* experiments demonstrated that the action of BPA was equally effective as that of E2 and that it was directly on beta-cells. The use of ERalpha-/- mice showed that the action of both E2 and BPA was ERalpha mediated in a non-classical manner (48).

In physiological conditions such as pregnancy or puberty, estrogen signaling will cause beta-cells to overwork so as to counteract the peripheral insulin resistance during these metabolic states (19). However, overactivation of estrogen signaling by an environmental estrogen such as BPA will produce excessive insulin signaling in an organism that does not need this extra insulin signaling. This situation may provoke insulin resistance in the liver and skeletal muscle, as well as beta-cell exhaustion and death, thereby possibly contributing to the development of type 2 diabetes (9).

9. CONCLUSIONS

Experiments published during the last decade have demonstrated that beta-cells are important targets for estrogen signaling. They express both classical ERs. ERalpha triggers the E2-regulation of insulin content and secretion and has a prominent role in beta-cell cytoprotection. The activation of ERbeta regulates stimulus-secretion coupling in beta-cells provoking a rapid insulin release (Figure 4). In addition, it has a minor role in preventing apoptosis. Importantly, ERalpha and ERbeta actions occur at physiological doses of E2 found, for instance, during pregnancy. Finally, GPER1 is also expressed in the three different types of cells presented in the islets. It is involved in the E2-induced potentiation of insulin release at supraphysiological doses of E2. The GPER1 agonist G-1

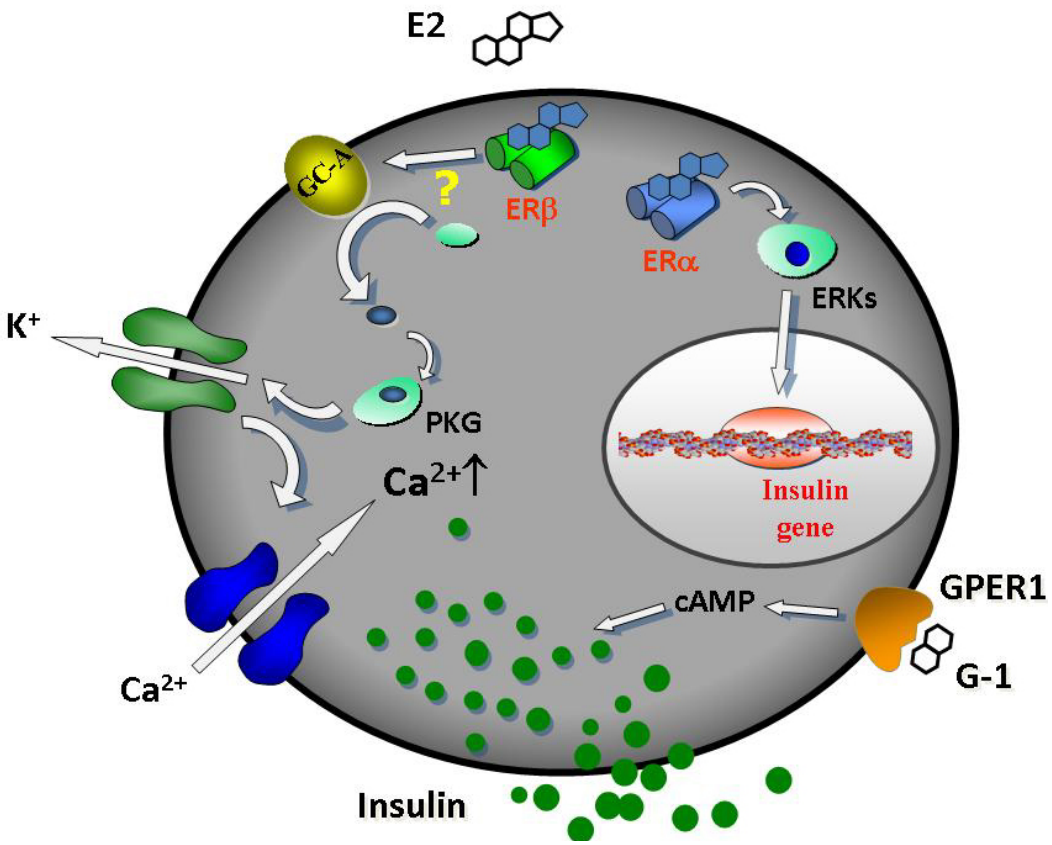

Figure 4. Model for the role of ERalpha, ERbeta and GPER1 in E2 regulation of insulin content and secretion. In synergy with glucose, binding of E2 to ERbeta elicits a blockade of KATP channels via activation of the GC-A receptor. The closure of KATP channels will produce a depolarization of the plasma membrane, an opening of voltage gated Ca2+ channels and a potentiation of insulin release. The activation of extranuclear ERalpha regulates insulin expression and content via an ERK1/2 dependent mechanism. The GPER1 agonist G-1 has an insulinotropic effect, most likely mediated by cAMP. G-1 mimics E2 action although with lesser efficacy.

enhances glucose-induced insulin secretion with less efficacy than E2, yet it is equally effective in regulating stimulus secretion coupling in alpha- and delta-cells. In addition, GPER1 has an antiapoptotic action in beta-cells. The role that estrogen receptors may have in alpha and delta-cells as well as the paracrine function exerted on beta-cells are an interesting line for future research.

10. ACNOWLEGDMENTS

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