PLOS ONE: Rapid Insulinotropic Action of Low Doses of Bisphenol-A on Mouse and Human Islets of Langerhans: Role of Estrogen Receptor β

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Abstract

Bisphenol-A (BPA) is a widespread endocrine-disrupting chemical (EDC) used as the base compound in the manufacture of polycarbonate plastics. It alters pancreatic β-cell function and can be considered a risk factor for type 2 diabetes in rodents. Here we used ERβ−/− mice to study whether ERβ is involved in the rapid regulation of K_{ATP} channel activity, calcium signals and insulin release elicited by environmentally relevant doses of BPA (1 nM). We also investigated these effects of BPA in β-cells and whole islets of Langerhans from humans. 1 nM BPA rapidly decreased K_{ATP} channel activity, increased glucose-induced $[Ca^{2+}]_i$ signals and insulin release in β-cells from WT mice but not in cells from ERβ−/− mice. The rapid reduction in the K_{ATP} channel activity and the insulinotropic effect was seen in human cells and islets. BPA actions were stronger in human islets compared to mouse islets when the same BPA concentration was used. Our findings suggest that BPA behaves as a strong estrogen via nuclear ERβ and indicate that results obtained with BPA in mouse β-cells may be extrapolated to humans. This supports that BPA should be considered as a risk factor for metabolic disorders in humans.

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Introduction

Bisphenol-A (BPA) is a widespread endocrine disruptor that produces insulin resistance and alterations in pancreatic β-cell function [1]. It has been suggested that together with other endocrine disrupting chemicals (EDCs), BPA constitutes a risk factor for type 2 diabetes and other metabolic disorders [2]–[4]. Recent work shows the association between increasing urinary BPA levels and Diabetes Mellitus. A 12.8% of diabetic patients show high BPA levels in urine samples (4.20 ng/ml or 18 nM) [5]. Moreover an association between urinary levels of BPA, obesity and insulin resistance in middle-aged and elderly chinese adults has been recently described [6]. The range of BPA levels found in humans is from 0.7 to 20 nM [7], [8]. To support the evidence that BPA may be a risk for the development of diabetes it is critical to study its effect on human tissues involved in glucose and lipid metabolism, including the endocrine pancreas which is key in glucose homeostasis.

The islet of Langerhans is the physiological unit of the endocrine pancreas; it is a group of 1500–3000 cells of five different types [9]–[11], and the most abundant are β-cells. The main function of β-cells is the biosynthesis and release of insulin in response to neurotransmitters, hormones and nutrients, the most important being glucose. The secretory response of β-cells depends on their electrical activity. This consists of oscillations of the membrane potential that range from electrically silent periods to depolarized plateaus on which Ca^{2+}-action potential originate [12]. Classically, stimulus-secretion coupling involves the closure of K_{ATP} channels after an increase in the ATP/ADP ratio because of the glucose metabolism [13]. K_{ATP} channels are responsible for the resting membrane potential of β-cells and its closure elicits a depolarization that opens voltage dependent calcium channels and induces Ca^{2+} influx [14]. As a consequence of the oscillatory membrane potential, a $[Ca^{2+}]_i$ oscillatory pattern originates [15]–[19], which triggers a pulsatile insulin secretion [17]–[19]. In addition to the K_{ATP} dependent process of insulin secretion there is a K_{ATP} independent process which involves cAMP dependent phosphorylation [20], [21].

Beta cells express estrogen receptor α (ERα), estrogen receptor β (ERβ) and the G-protein coupled receptor (GPR30), also named GPER1 [22], [23]. The use of genetically modified mice has revealed the role of these estrogen receptors [23]. ERα is involved in the regulation of pancreatic insulin biosynthesis in response to both E2 and BPA. Remarkably, nanomolar concentrations of either BPA or E2 act via extranuclear ERα to activate ERK1/2 and regulate insulin content [24]. This action involves the activation of the transcription factor NeuroD1 [25]. In addition, activation of extranuclear ERβ by physiological concentrations of E2 rapidly regulates K_{ATP} channel activity, increases glucose stimulated $[Ca^{2+}]_i$ signals and insulin release. It is important to clarify that action of E2 on K_{ATP} channel activity was mimicked by specific agonist of ERβ 2,3-bis (4-hydroxyphenyl)-propionitrile (DPN) but not by the ERα specific agonist propylpyrazole-triol (PPT). Moreover K_{ATP} channel activity was not modified in ERα−/− [26]. These results indicated that ERβ plays an important role in rapid regulation of insulin secretion in pancreatic β-cells. Bisphenol-A imitated rapid estradiol regulation of K_{ATP} channel and calcium signaling [1], however, whether ERβ acting out of the nucleus is able to mediate BPA actions is still unknown.

In the present work we use β-cells and islets of Langerhans from wild type (WT) and ERβ−/− mice to show that ERβ is involved in the BPA-mediated rapid regulation of K_{ATP} channel activity, potentiation of glucose induced-$[Ca^{2+}]_i$ signals and insulin release. Moreover, we have used human islets of Langerhans to demonstrate that 1 nM BPA blocked K_{ATP} channels and produced a potent enhancement of insulin secretion in response to glucose.

Materials and Methods

Animals

Adult C57 female mice were used. All animals were kept under standard housing conditions. The ethical

committee of Miguel Hernandez University “Comisión de Ética en la Investigación Experimental” reviewed and approved the method used. ERβ−/−mice were generated as described prior [27] and supplied by Jan-Ake Gustafsson's laboratory. All genetically modified animals and wild types were from the same supplier and the same colony. Islets of Langerhans from ERβ−/− mice were treated as described below for islets from C57 mice. Animals were kept in new polycarbonate cages and polycarbonate water bottles were avoided to minimize contamination of mice with free BPA.

Islet of Langerhans isolation from mice

Adult mice were killed by cervical dislocation and islets were isolated with a collagenase technique previously described [26] and used according to the kind of experiment to be performed.

Human islets isolation for electrophysiological experiments

Human pancreases were obtained from 3 cadaveric organ donors, after written consent of their families and approval of “Comité Ético de Investigación Clínica”, and “Comité de Investigación”, Hospital Clínic of Barcelona (ID: 2009/5157). Islets were isolated by collagenase digestion of the pancreas (SERVA Electrophoresis, Heidelberg, Germany) and separated from exocrine tissue by Biocoll density gradient (Biochrom, Berlin,Germany), as previously described [28]. Islets were transferred to RPMI-1640 medium (Gibco-BRL, Pisley, U.K.) containing 11.1 mmol/l glucose and supplemented with 10% FBS, 2 mmol/l L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin and cultured overnight at 37°C with 5% CO2. Islets were then picked and disaggregated to obtain beta cells for electrophysiological studies as described below.

Obtaining β-cells from mouse and human islets

Once mouse or human islets were obtained as described above, they were dispersed into single cells and cultured as previously described [14]. The protocol of islet disaggregation into sinlge cells was the same for both mouse and human islets. Once isolated, islets were disaggregated into single cells in a low calcium medium. Cells were then centrifuged, resuspended in culture medium RPMI 1640 without phenol red containing 11 mM glucose supplemented with 10% charcoal dextran-treated fetal bovine serum (HyClone Laboratories, Logan, UT), 2 mM L-glutamine, 200 U/mL penicillin and 0.2 mg/mL streptomycin and plated on glass coverslips. Cells were kept at 37°C, in a humidified atmosphere of 95% O_2 and 5% CO_2, and used within 1–2 days of culture.

Insulin secretion measurements in mouse islets

Freshly isolated islets of Langerhans from mice were left to recover in the isolation medium for 2 h in the incubator. After recovery, groups of 5 were transferred to 400 µl of a buffer solution containing 140 mM NaCl, 4.5 mM KCl, 2.5 mM CaCl2, 1 mM MgCl2, 20 mM HEPES and the glucose concentration corresponding to each experimental condition with final pH at 7.4. Afterwards, 100 µl of the corresponding buffer solution with 5% Bovine Serum Albumin (BSA) was added, incubated at room temperature for 3 min and let to cool down for 15 min on ice. Then, the medium was collected and insulin was measured in duplicate samples by radioimmunoassay using a Coat-a-Count kit (Siemens, Los Angeles, CA, USA).

Recording intracellular calcium concentration ($[Ca^{2+}]_i$)

Freshly isolated islets of Langerhans from mice were loaded with 5 µM Fura-2 AM for at least 1 h at room temperature. Calcium recordings in islets were obtained by imaging intracellular calcium under an inverted epifluorescence microscope (Zeiss, Axiovert 200). Images were acquired every 2 s with an extended Hamamatsu Digital Camera C4742-95 (Hamamatsu Photonics, Barcelona, Spain) using a dual filter wheel

(Sutter Instrument CO, CA, USA) equipped with 340 nm and 380 nm, 10 nm bandpass filters (Omega optics, Madrid, Spain). Data were acquired using Aquacosmos software from Hamamatsu (Hamamatsu Photonics, Barcelona, Spain). Results were plotted and analyzed using commercially available software (Sigmaplot, Jandel Scientific). Data were represented as a frequency of calcium oscillations (min^{-1}) comparing BPA responses with their respective control.

Patch Clamp recordings

K_{ATP} channel activity was recorded using standard patch clamp recording procedures from isolated β-cells [29]. 80–90% of the single cells were identified as β-cells by their large size (capacitance: 8–12 pF) and by their electrophysiological properties, such as presence of K_{ATP} channel activity in absence of glucose and their response to glucose by the appearance of action currents in cell-attached configuration. Currents were recorded using an Axopatch 200B patch-clamp amplifier (Axon Instruments Co. CA, USA). Patch pipettes were pulled from borosilicate capillaries (Sutter Instruments Co. CA, USA) using a flaming/brown micropipette puller P-97 (Sutter Instruments Co. CA, USA) with resistance between 3–5 MΩ when filled with the pipette solutions as specified below. Bath solution contained (in mM): 5 KCl, 135 NaCl, 2.5 $CaCl_2$, 10 Hepes and 1.1 $MgCl_2$ (pH 7.4) and supplemented with glucose as indicated. The pipette solution contained (in mM): 140 KCl, 1 $MgCl_2$, 10 Hepes and 1 EGTA (pH 7.2). The pipette potential was held at 0 mV throughout recording. K_{ATP} channel activity was quantified by digitising 60 s sections of the current record, filtered at 1 kHz, sampled at 10 kHz by a Digidata 1322A (Axon Instruments Co. CA, USA), and calculating the mean open probability of the channel (NP_o) during the sweep. Channel activity was defined as the product of N, the number of functional channels, and P_o, the open-state probability. P_o was determined by dividing the total time channels spent in the open state by the total sample time. Data were represented as a percentage of activity with respect to resting conditions (0 mM Glucose). Experiments were carried out at room temperature (20–24°C).

Human pancreatic islets and measurement of human islet insulin secretion

Isolated human pancreatic islets from non-diabetic males (age 58+6 years; Hb1Ac<6.1 and MBI: 27+2) were provided by the Nordic network for clinical islet transplantation (Professor Olle Korsgren, Uppsala University, Sweden). All procedures were approved by the ethical committees at Uppsala and Lund Universities. Prior to the experiments the islets had been cultured at 37°C (5% CO_2) for 2 days in CMRL 1066 (ICN Biomedicals, Costa Mesa, CA) supplemented with 10 mmol/l HEPES, 2 mmol/l L-glutamine, 50 μg/ml gentamicin, 0.25 μg/ml Fungizone (Gibco, BRL, Gaithersburg, MD), 20 μg/ml ciprofloxacin (Bayer Healthcare, Leverkusen, Germany) and 10 mmol/l nicotinamide. The islets had 70–90% purity when they arrived; the islets were then hand-picked under stereomicroscope prior to use. All islet preparations were treated in exactly the same way to avoid results being due to handling differences. All procedures were approved by the ethical committees at Uppsala and Lund Universities.

At the experiment day, the islets were preincubated for 30 min at 37°C in Krebs-Ringer bicarbonate buffer, pH 7.4, supplemented with 10 mmol/l HEPES, 0.1% bovine serum albumin and 1.0 mmol/l glucose. After preincubation the buffer was changed and the islets were incubated at 8.3 mmol/l glucose ± different test agents for 60 min at 37°C. Each incubation tube contained 12 islets in 1.0 ml of KRB solution and was gassed with 95% O_2 5% CO_2 to obtain constant pH and oxygenation. All incubations were performed in an incubation box at 30 cycles/min. immediately after incubation an aliquot of the medium was removed and frozen for subsequent assay of insulin. The secreted insulin was measured using a radioimmunoassay kit (Millipore).

Results

BPA action on K_{ATP} channel activity is abolished in β-cells from ERβ−/− mice

Bisphenol-A application in presence of 8 mM glucose during 1 hour increased insulin release from mouse islet of Langerhans in a dose dependent manner (Figure 1A). For the rest of the work we used 1 nM BPA because is within the range of BPA levels in human serum and it is considered an environmentally relevant dose [7], [8].

Figure 2A shows, in a typical β-cell from wt mice, the basal activity of K_{ATP} channels (upper trace). Channel openings are represented by downward deflections, reflecting inward currents due to the high potassium content of the pipette. The criterion used for identification of K_{ATP} channels was their sensitivity to glucose, which highly decreases channel activity (Fig. 2A, 8 mM glucose trace) and diazoxide, a sulfonamide that opens K_{ATP} channels (Fig. 2A, 100 uM diazoxide trace). Both glucose and diazoxide sensitivity were tested in all records throughout this work. Figure 2A (BPA trace) demonstrates the decrease in the K_{ATP} channel activity elicited by BPA, 7 min after its application. On average, BPA decreases K_{ATP} channel activity by 49.3% (Fig. 2B).

Figure 2. K_{ATP} channel activity in pancreatic β-cells from WT and ERβ−/− mice in presence of 1 nM BPA.

A, BPA at 1 nM decreased K_{ATP} channel activity in pancreatic β-cells from WT mice. The records show channel activity before application of BPA (upper trace), 7 min after application of BPA (1 nM BPA trace), 8 min after application of 8 mM Glucose (8 mM glucose trace) and 2 min after application of diazoxide (100 μM diazoxide trace) (n = 7). B, percentage of activity of the K_{ATP} channels elicited by vehicle and 1 nM BPA. C, 1 nM BPA had no significant effect on K_{ATP} channel activity in pancreatic ERβ−/− mice. As in A, the records show K_{ATP} channel activity before application of BPA, 7 min after application of 1 nM BPA, 8 min after application of 8 mM glucose and 3 min after application of 100 μM diazoxide (n = 5 cells for 3 different mice). D, percentage of activity of K_{ATP} channels elicited by vehicle and 1 nM BPA. *, $P<0.05$ Student's test comparing 1 nM BPA with control.

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To molecularly study the role of ERβ in BPA-induced rapid regulation of K_{ATP} channels, cell-attached patch recordings were performed in β-cells from ERβ−/− mice. In these cells K_{ATP} channel activity was not significantly altered after BPA application (Fig. 2C and D).

BPA closes K_{ATP} channels in human β-cells

In order to analyze whether the rapid action of BPA on K_{ATP} channel activity occurred as well in human pancreatic β-cells, islets from human donors were disaggregated into single cells and single channel recordings were performed using the patch-clamp technique as performed in the previous subheading. Figure 3A shows a typical record in which 5 min after BPA application in the absence of glucose, there is a dramatic decrease in K_{ATP} channel activity by 83.4%, together with a depolarization of the membrane noticed by the decrease in the amplitude of the channel opening and the appearance of biphasic spikes (see * and ** in second and third traces and figure 3B). These biphasic spikes (action currents) were seen in all human cells exposed to either BPA or glucose and are action potentials seen via the capacitance and resistance of the patch and their recordings depend on the patch resistance [14], [30]. Remarkably, the response to BPA in the average of experiments is as high as that of 8 mM glucose (Fig. 3C). The action of BPA was mimicked by the ERβ agonist diarylpropionitrile (DPN) (Figure S1).

BPA action on glucose induced $[Ca^{2+}]_i$ oscillations and insulin release is abolished in

islets from ERβ−/− mice

It is well established that E2 rapidly induces Ca^{2+} signals and insulin secretion via ERβ in a process that depends on electrical activity [26]. To investigate whether activation of ERβ by BPA potentiated glucose-induced $[Ca^{2+}]_i$ signals and insulin secretion, we performed the experiments described in Figures 4 and 5. Islets loaded with the calcium fluorescence dye Fura-2 were imaged to monitor the $[Ca^{2+}]_i$ signal elicited by a change from a non stimulatory glucose concentration (3 mM) to an insulin stimulatory glucose concentration (8 mM). The response, as expected, was a $[Ca^{2+}]_i$ transient followed by a plateau with $[Ca^{2+}]_i$ oscillations. In the absence of BPA the frequency of $[Ca^{2+}]_i$ oscillations was similar in islets isolated from wt and ERβ−/− mice (Fig. 4A and C). However the frequency of $[Ca^{2+}]_i$ oscillations increased in islets exposed to 1 nM BPA compared to control in wt islets (Fig. 4B and C). It is of note that the BPA-induced increase in $[Ca^{2+}]_i$ oscillatory frequency is highly diminished in islets from ERβ−/− animals (Figure 4C). This experiment indicates that ERβ is involved in the BPA potentiation of glucose induced oscillatory activity.

In addition, BPA enhanced insulin release stimulated by 8 mM glucose islets from wt animals (Fig. 5A) but it had no effect in islets from ERβ−/− mice (Fig. 5B). Note that BPA had an effect only when a stimulatory glucose concentration (8 mM) was used; when BPA was applied together with 3 mM glucose no effect was observed.

Insulinotropic action of BPA on human islets of Langerhans

The rapid effect of 1 nM BPA on glucose stimulated insulin release was studied on human islets from two different donors. Islets responded to 8 mM glucose with an increase of insulin secretion. The action of 8 mM glucose was enhanced almost 2 fold by the presence of 1 nM BPA (Fig. 6). The ERβ agonist DPN applied at 1 nM concentration also enhanced insulin secretion, but to a lesser extent than 1 nM BPA (Fig. 6). This experiment indicates that environmentally relevant doses of BPA have an insulinotropic action on human islets of Langerhans.

Discussion

The findings of this work using β-cells and whole islets of Langerhans from human donors demonstrate that the widespread endocrine disruptor BPA induces a rapid decrease of the activity of K_{ATP} channels, a key molecule in the stimulus secretion coupling of β-cells. Moreover, the present work demonstrates that environmentally relevant doses of BPA (1 nM) stimulated glucose-induced insulin secretion in human islets, giving a response which is almost twice the insulin release elicited by a stimulatory glucose concentration, 8 mM. These results together with previous observations that BPA diminished adiponectin release in human adipocytes [31], [32] support the premise that BPA has adverse effects on glucose metabolism in adult humans. Additionally, epidemiological evidence links BPA levels in urine with metabolic disorders in adult humans. These include type-2 diabetes, cardiovascular diseases and insulin resistance [33], [34].

In adult rodents, BPA exposure at environmentally relevant doses provokes insulin resistance and hyperinsulinemia in the fed state [35]. The hyperinsulinemia is not just a consequence of insulin resistance, because BPA directly regulates pancreatic insulin content and insulin release in isolated islet of Langerhans from the mouse [24]. Remarkably, this action occurred at low concentrations of BPA (1 nM) via activation of extranuclear ERα in a nonclassical manner that involves ERK1/2 activation and a nonmonotonic dose response [24]. The rapid effect described in the present work using β-cells from ERβ−/− mice demonstrates that ERβ is involved in the inhibition of K_{ATP} channel activity and in the

regulation of glucose-induced $[Ca^{2+}]_i$ signals and insulin release. These actions imitate those of the natural hormone E2, and BPA and E2 are equally potent at 1 nM. It must be noted, however, that experiments performed in figures 2 and 4 using cells from ERβ−/− mice show a trend toward reduction in K_{ATP} channel activity and increased calcium signaling. In addition to ERα and ERβ, other non-classical membrane estrogen receptors (ncmER) involved in rapid BPA action have been described in β and α-cells [1], [23], [36]. GPR30 may be one of this ncmER because it exists in β-cells and is involved in cell survival [22], [37] and in glucose stimulated insulin secretion in response to supraphysiological concentrations of 17β-estradiol [22], [38]. Moreover, ncmERs may play a role in the supression of adiponectin release by BPA in mouse and human adipocytes [32].

BPA has been considered a weak estrogen because of its low binding affinity to both ERα and ERβ [39], as well as a low transcriptional activity through these ERE binding receptors [40]. ERα and ERβ control nuclear processes that include proliferation, apoptosis and migration exerting opposite effects in different tissues [41]. Nevertheless, it is fully accepted today that estrogen receptors do not work only in this classical manner but they signal as wellfrom extranuclear locations to exert a plethora of actions in different types of cells [37], [42]–[45]. In β-cells non-classical estrogen triggered pathways include regulation of insulin biosynthesis by ERα [24], [25], regulation of glucose stimulated insulin release by ERβ [26], improvement of cell survival by ERα and to a lesser extent by ERβ and GPR30 [37] and control of lipid synthesis by extranuclear ERα [45]. The nonclassical actions of estrogen receptors have main implications in energy balance as demonstrated using genetic rescue of nonclassical ER signalling in ER−/−mice [46]. The use of ERβ−/− mice in the present work and ERα−/− mice in a previous work [24] unequivocally demonstrates that both receptors acting though nonclassical pathways mediate BPA and E2 actions at equal concentrations (Fig. 7). Therefore, BPA cannot be considered a weak estrogen anymore, at least not in β-cells. Nevertheless, why the BPA effects occur at low doses when ERs are located in the extranuclear compartment is a matter of future research. It is possible that BPA binds differently to ERα and ERβ when they are located outside the nucleus. Also the recruitment of co-activators or co-repressors may be completely different [47]. Some of these effects may even be cell-specific. For instance, the insulinotropic action of BPA shown in this work occurs in the presence of a stimulatory glucose concentration of 8 mM. Under this condition most K_{ATP} channels are already closed and therefore the membrane resistance is high, so that a small current will elicit the depolarization of the membrane, electrical activity, Ca^{2+} signals and insulin release. This occurs in β-cells because K_{ATP} channels control the resting membrane potential and determine the electrical resistance of the cell [48]. This implies that the closure of a small number of K_{ATP} channels by BPA will produce an insulinotropic action.

In the case of human islets, BPA had a similar action to that in mice. It elicited a decrease in the K_{ATP} channel activity which was stronger than that in mice when the same dose of BPA was used. Moreover, its insulinotropic action was much stronger in human cells that in mice at the same dose. These results clearly demonstrate that environmentally relevant doses of BPA (1 nM) have an insulinotropic action on human islets. It is of note that the concentrations of BPA found in humans range from 0.7 to 20 nM [7], [8] and therefore the effect demonstrated here with islets from human donors may occur *in vivo* at usual levels of exposure. Rapid release of insulin *in vivo* in response to 10 µg/kg BPA was already reported in mice [35]. An excessive insulin signalling produced by an overstimulation of β-cells by BPA exposure may produce dyslipidemia resulting from effects in the liver and adipose tissue, and obesity and glucose intolerance [49]. In addition it may provoke insulin resistance in liver and skeletal muscle together with β-cell exhaustion, contributing to the development of type-2 diabetes [1]. Alterations of glucose and lipid metabolism by BPA in adults may constitute a significant hazard during pregnancy for both mothers and offspring as demonstrated in mice and rats [50], [51].

The ERβ agonist DPN also closed K_{ATP} channels and rapidly induced insulin release in human β-cells but to a smaller extent compared to mice. Given the scarcity of human islets we do not have enough data to

definitely conclude that ERβ is the main receptor involved. The fact that ERβ mediates the insulinotropic action of BPA in mice does not rule out the possibility that other receptors such as GPR30/GPER1, which also bind BPA, may have different actions in mice and humans (fig. 7). Using GPR30 KO mice, it has recently been demonstrated that in addition to ERβ, GPR30 may also participate in the insulinotropic action of E2 in mice [38]. Moreover, it is known that activation of GPR30 by its agonist G1 is insulinotropic in mouse and human islets [22], [52] and regulates glucagon and somatostatin release from α and δ cells within the islet of Langerhans. Other not yet identified receptors acting on β- and other types of cells may also have a role in BPA signalling [31], [32], [53], [54].

The use of β-cells from genetically modified mice indicates that BPA cannot longer be considered a weak estrogen because it is equally potent to the natural hormone E2 when acting via nonclassical ER-mediated pathways. The rapid insulinotropic action of BPA described in mice also occurs in human islets and in a stronger manner to that in mice. This should have important implications for the policy of exposure of humans to BPA. There is an ongoing debate among environmental agencies worldwide about whether BPA can be considered a hazard for human health. One of the constant points of discussion is the relevance of extrapolation of animal results to humans. The demonstration that BPA at concentrations found in human serum alters human β-cell function strongly indicates that at least some of the adverse effects of BPA on glucose homeostasis described in mice may be translated to humans.

Supporting Information

Figure S1.

Activation of ERβ by DPN decreased K_{ATP} channel activity in human pancreatic β-cells. 1 nM DPN, specific agonist of ERβ, decreased K_{ATP} channel activity in isolated human pancreatic β-cells (1 nM DPN trace) compared with control conditions at 0 mM glucose concentration (vehicle trace). (n=2).

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(TIF)

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Author Contributions

Conceived and designed the experiments: SS PA-M IQ A. Nadal. Performed the experiments: SS PA-M MG-A SM. Analyzed the data: SS PA-M MG-A SM AS A. Nadal. Contributed reagents/materials/analysis tools: A. Novials AS JAG IQ A. Nadal. Wrote the paper: A. Nadal.

References

1. 1. Nadal A, Alonso-Magdalena P, Soriano S, Quesada I, Ropero AB (2009) The pancreatic beta-cell as a target of estrogens and xenoestrogens: Implications for blood glucose homeostasis and diabetes. Mol Cell Endocrinol 304: 63–68.
2. 2. Alonso-Magdalena P, Quesada I, Nadal A (2011) Endocrine disruptors in the etiology of type 2 diabetes mellitus. Nat Rev Endocrinol 7: 346–353.
3. 3. Hectors TL, Vanparys C, van der Ven K, Martens GA, Jorens PG, et al. (2011) Environmental pollutants and type 2 diabetes: a review of mechanisms that can disrupt beta cell function.

Diabetologia 54: 1273–1290.

4. 4. Neel BA, Sargis RM (2011) The paradox of progress: environmental disruption of metabolism and the diabetes epidemic. Diabetes 60: 1838–1848.
5. 5. Shankar A, Teppala S (2011) Relationship between Urinary Bisphenol A Levels and Diabetes Mellitus. J Clin Endocrinol Metab 96: 3822–3826.
6. 6. Wang T, Li M, Chen B, Xu M, Xu Y, et al. (2011) Urinary Bisphenol A (BPA) Concentration Associates with Obesity and Insulin Resistance. J Clin Endocrinol Metab. doi:10.1210/jc.2011-1989.
7. 7. vom Saal FS, Nagel SC, Timms BG, Welshons WV (2005) Implications for human health of the extensive bisphenol A literature showing adverse effects at low doses: a response to attempts to mislead the public. Toxicology 212: 244–252, author reply 253–244.
8. 8. vom Saal FS, Akingbemi BT, Belcher SM, Birnbaum LS, Crain DA, et al. (2007) Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. Reprod Toxicol 24: 131–138.
9. 9. Brissova M, Fowler MJ, Nicholson WE, Chu A, Hirshberg B, et al. (2005) Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. J Histochem Cytochem 53: 1087–1097.
10. 10. Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO, et al. (2006) The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. Proc Natl Acad Sci U S A 103: 2334–2339.
11. 11. Prado CL, Pugh-Bernard AE, Elghazi L, Sosa-Pineda B, Sussel L (2004) Ghrelin cells replace insulin-producing beta cells in two mouse models of pancreas development. Proc Natl Acad Sci U S A 101: 2924–2929.
12. 12. Rorsman P, Eliasson L, Renstrom E, Gromada J, Barg S, et al. (2000) The Cell Physiology of Biphasic Insulin Secretion. News Physiol Sci 15: 72–77.
13. 13. Ashcroft FM, Harrison DE, Ashcroft SJ (1984) Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. Nature 312: 446–448.
14. 14. Valdeolmillos M, Nadal A, Contreras D, Soria B (1992) The relationship between glucose-induced K+ATP channel closure and the rise in [Ca2+]i in single mouse pancreatic beta-cells. J Physiol 455: 173–186.
15. 15. Nadal A, Quesada I, Soria B (1999) Homologous and heterologous asynchronicity between identified alpha-, beta- and delta-cells within intact islets of Langerhans in the mouse. J Physiol 517(Pt 1): 85–93.
16. 16. Santos RM, Rosario LM, Nadal A, Garcia-Sancho J, Soria B, et al. (1991) Widespread synchronous [Ca2+]i oscillations due to bursting electrical activity in single pancreatic islets. Pflugers Arch 418: 417–422.
17. 17. Barbosa RM, Silva AM, Tome AR, Stamford JA, Santos RM, et al. (1998) Control of pulsatile 5-HT/insulin secretion from single mouse pancreatic islets by intracellular calcium dynamics. J Physiol 510(Pt 1): 135–143.
18. 18. Dyachok O, Idevall-Hagren O, Sagetorp J, Tian G, Wuttke A, et al. (2008) Glucose-induced cyclic AMP oscillations regulate pulsatile insulin secretion. Cell Metab 8: 26–37.
19. 19. Gilon P, Shepherd RM, Henquin JC (1993) Oscillations of secretion driven by oscillations of cytoplasmic Ca2+ as evidences in single pancreatic islets. J Biol Chem 268: 22265–22268.
20. 20. Sato Y, Henquin JC (1998) The K+-ATP channel-independent pathway of regulation of insulin

secretion by glucose: in search of the underlying mechanism. Diabetes 47: 1713–1721.

21. 21. Straub SG, James RF, Dunne MJ, Sharp GW (1998) Glucose activates both K(ATP) channel-dependent and K(ATP) channel-independent signaling pathways in human islets. Diabetes 47: 758–763.
22. 22. Balhuizen A, Kumar R, Amisten S, Lundquist I, Salehi A (2010) Activation of G protein-coupled receptor 30 modulates hormone secretion and counteracts cytokine-induced apoptosis in pancreatic islets of female mice. Mol Cell Endocrinol 320: 16–24.
23. 23. Nadal A, Alonso-Magdalena P, Soriano S, Ripoll C, Fuentes E, et al. (2011) Role of estrogen receptors alpha, beta and GPER1/GPR30 in pancreatic beta-cells. Front Biosci 16: 251–260.
24. 24. Alonso-Magdalena P, Ropero AB, Carrera MP, Cederroth CR, Baquie M, et al. (2008) Pancreatic insulin content regulation by the estrogen receptor ER alpha. PLoS One 3: e2069.
25. 25. Wong WP, Tiano JP, Liu S, Hewitt SC, Le May C, et al. (2010) Extranuclear estrogen receptor-alpha stimulates NeuroD1 binding to the insulin promoter and favors insulin synthesis. Proc Natl Acad Sci U S A 107: 13057–13062.
26. 26. Soriano S, Ropero AB, Alonso-Magdalena P, Ripoll C, Quesada I, et al. (2009) Rapid regulation of K(ATP) channel activity by 17{beta}-estradiol in pancreatic {beta}-cells involves the estrogen receptor {beta} and the atrial natriuretic peptide receptor. Mol Endocrinol 23: 1973–1982.
27. 27. Krege JH, Hodgin JB, Couse JF, Enmark E, Warner M, et al. (1998) Generation and reproductive phenotypes of mice lacking estrogen receptor beta. Proc Natl Acad Sci U S A 95: 15677–15682.
28. 28. Novials A, Sarri Y, Casamitjana R, Rivera F, Gomis R (1993) Regulation of islet amyloid polypeptide in human pancreatic islets. Diabetes 42: 1514–1519.
29. 29. Ropero AB, Fuentes E, Rovira JM, Ripoll C, Soria B, et al. (1999) Non-genomic actions of 17beta-oestradiol in mouse pancreatic beta-cells are mediated by a cGMP-dependent protein kinase. J Physiol 521 Pt 2: 397–407.
30. 30. Fenwick EM, Marty A, Neher E (1982) A patch-clamp study of bovine chromaffin cells and of their sensitivity to acetylcholine. J Physiol 331: 577–597.
31. 31. Ben-Jonathan N, Hugo ER, Brandebourg TD (2009) Effects of bisphenol A on adipokine release from human adipose tissue: Implications for the metabolic syndrome. Mol Cell Endocrinol 304: 49–54.
32. 32. Hugo ER, Brandebourg TD, Woo JG, Loftus J, Alexander JW, et al. (2008) Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. Environ Health Perspect 116: 1642–1647.
33. 33. Lang IA, Galloway TS, Scarlett A, Henley WE, Depledge M, et al. (2008) Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. Jama 300: 1303–1310.
34. 34. Melzer D, Rice NE, Lewis C, Henley WE, Galloway TS (2010) Association of urinary bisphenol a concentration with heart disease: evidence from NHANES 2003/06. PLoS One 5: e8673.
35. 35. Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A (2006) The estrogenic effect of bisphenol A disrupts pancreatic beta-cell function in vivo and induces insulin resistance. Environ Health Perspect 114: 106–112.
36. 36. Alonso-Magdalena P, Laribi O, Ropero AB, Fuentes E, Ripoll C, et al. (2005) Low doses of bisphenol A and diethylstilbestrol impair Ca2+ signals in pancreatic alpha-cells through a nonclassical membrane estrogen receptor within intact islets of Langerhans. Environ Health

Perspect 113: 969–977.

37. 37. Liu S, Le May C, Wong WP, Ward RD, Clegg DJ, et al. (2009) Importance of extranuclear estrogen receptor-alpha and membrane G protein-coupled estrogen receptor in pancreatic islet survival. Diabetes 58: 2292–2302.
38. 38. Sharma G, Prossnitz ER (2011) Mechanisms of estradiol-induced insulin secretion by the G protein-coupled estrogen receptor GPR30/GPER in pancreatic beta-cells. Endocrinology 152: 3030–3039.
39. 39. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, et al. (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 139: 4252–4263.
40. 40. Sheeler CQ, Dudley MW, Khan SA (2000) Environmental estrogens induce transcriptionally active estrogen receptor dimers in yeast: activity potentiated by the coactivator RIP140. Environ Health Perspect 108: 97–103.
41. 41. Thomas C, Gustafsson JA (2011) The different roles of ER subtypes in cancer biology and therapy. Nat Rev Cancer 11: 597–608.
42. 42. Levin ER (2010) Minireview: Extranuclear steroid receptors: roles in modulation of cell functions. Mol Endocrinol 25: 377–384.
43. 43. Nadal A, Diaz M, Valverde MA (2001) The estrogen trinity: membrane, cytosolic, and nuclear effects. News Physiol Sci 16: 251–255.
44. 44. Liu S, Mauvais-Jarvis F (2009) Rapid, nongenomic estrogen actions protect pancreatic islet survival. Islets 1: 273–275.
45. 45. Tiano JP, Delghingaro-Augusto V, Le May C, Liu S, Kaw MK, et al. (2011) Estrogen receptor activation reduces lipid synthesis in pancreatic islets and prevents beta cell failure in rodent models of type 2 diabetes. J Clin Invest 121: 3331–3342.
46. 46. Park CJ, Zhao Z, Glidewell-Kenney C, Lazic M, Chambon P, et al. (2011) Genetic rescue of nonclassical ERalpha signaling normalizes energy balance in obese ERalpha-null mutant mice. J Clin Invest 121: 604–612.
47. 47. Safe SH, Pallaroni L, Yoon K, Gaido K, Ross S, et al. (2002) Problems for risk assessment of endocrine-active estrogenic compounds. Environ Health Perspect 110: Suppl 6925–929.
48. 48. Ashcroft FM (2005) ATP-sensitive potassium channelopathies: focus on insulin secretion. J Clin Invest 115: 2047–2058.
49. 49. Biddinger SB, Kahn CR (2006) From mice to men: insights into the insulin resistance syndromes. Annu Rev Physiol 68: 123–158.
50. 50. Alonso-Magdalena P, Vieira E, Soriano S, Menes L, Burks D, et al. (2010) Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. Environ Health Perspect 118: 1243–1250.
51. 51. Wei J, Lin Y, Li Y, Ying C, Chen J, et al. (2011) Perinatal Exposure to Bisphenol A at Reference Dose Predisposes Offspring to Metabolic Syndrome in Adult Rats on a High-Fat Diet. Endocrinology 152: 3049–3061.
52. 52. Kumar R, Balhuizen A, Amisten S, Lundquist I, Salehi A (2011) Insulinotropic and Antidiabetic Effects of 17{beta}-Estradiol and the GPR30 Agonist G-1 on Human Pancreatic Islets. Endocrinology 152: 2568–2579.
53. 53. Nadal A, Ropero AB, Fuentes E, Soria B, Ripoll C (2004) Estrogen and xenoestrogen actions on endocrine pancreas: from ion channel modulation to activation of nuclear function. Steroids 69: 531–536.

54. 54. Bouskine A, Nebout M, Brucker-Davis F, Benahmed M, Fenichel P (2009) Low doses of bisphenol A promote human seminoma cell proliferation by activating PKA and PKG via a membrane G-protein-coupled estrogen receptor. Environ Health Perspect 117: 1053–1058.