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Review

Bisphenol-A: a new diabetogenic factor?

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ABSTRACT

The aim of this review was to analyze the potential effects of environmental chemicals on homeostatic control related to glycemia and energy balance. Many of the environmental chemicals can mimic or interfere with the action of hormones and are generally referred to as "endocrine disruptors". Among these compounds, polychlorinated biphenyls, dioxins, phthalates and bisphenol-A have been correlated with alterations in blood glucose homeostasis in humans. In rodents it has been demonstrated that small doses of bisphenol-A have profound effects on glucose metabolism. Therefore, this altered blood glucose homeostasis may enhance the development of type 2 diabetes.

Key words: Bisphenol-A, Diabetes, Estradiol, Estrogen Receptors, Glucose Homeostasis, Islet of Langerhans

1. FAILURE OF BLOOD GLUCOSE HOMEOSTASIS: THE ONSET OF DIABETES

Over three and a half millennia have passed since the first description of diabetes. An Egyptian papyrus of 1550 BC mentions a rare disorder that causes the patient to urinate frequently and to lose weight. Some centuries later, the Greek physician Aretaeus would name this condition "Diabetes mellitus", the first word meaning "a flowing through" and the complete appellation denoting the passing of large amounts of urine that is sweet because it contains sugar (glucose). Down through the ages different definitions were applied, but an in-depth understanding of the disease started to develop only during the last century.

Today, diabetes represents one of the most serious health problems worldwide. It has been estimated that more than 170 million people suffer from diabetes mellitus and this number is projected to increase to 366 million by the year 2030.[1] Despite constant efforts, the number of patients is increasing continuously and it is now estimated that diabetes is responsible for 2.9 million deaths per year.

While the etiology of the problem remains puzzling, what is well accepted is that two factors are crucial in the development of type 2 diabetes: insulin resistance and β-cell dysfunction. Moreover, it is thought that this disease has a multifactorial origin

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in which genetic predisposition, obesity, diet and lack of exercise seem to be important players.

How does diabetes appear?

In order to maintain blood glucose concentration within the physiological levels, a complex communication between different tissues, including brain, adipose tissue, muscle, liver and pancreas is required. In the fasting state, plasma glucose concentration is low, which in turn keeps plasma insulin levels low. By contrast, the levels of counter-regulatory hormones like glucagon, adrenaline and corticosteroids increase, this increase accounting for the production of glucose by the liver. On the other hand, after a meal, when the level of blood glucose is high, insulin is secreted by pancreatic β-cells. Insulin will decrease blood glucose by promoting glucose uptake by adipocytes and muscle, as well as preventing the liver from producing glucose by suppressing glycogenolysis and gluconeogenesis.[2-5]

Insulin sensitivity is a non-linear process and fluctuations occur during a normal life cycle. Thus diminished insulin sensitivity is observed during pregnancy, puberty or aging, which means that the efficiency of insulin to promote glucose uptake in muscle or fat or to inhibit glucose production in the liver is decreased. In normal conditions, this insulin resistance is compensated by an increase of insulin release by the pancreas, thereby maintaining normal glucose tolerance.[6,7] However, if this compensation fails, hyperglycemia will appear, which leads in turn to the development of type 2 diabetes.[6-9]

2. ESTRADIOL AS AN IMPORTANT HORMONE IMPLICATED IN THE CONTROL OF ENERGY AND BLOOD GLUCOSE HOMEOSTASIS

Current data indicate that estradiol (E2) is much more than a sex hormone, as it has been demonstrated for years that E2 plays an important role in the function of the cardiovascular, musculoskeletal, immune and central nervous systems.[10] Moreover, recent studies have shown the importance of E2 for energy balance and glucose homeostasis.[11,12]

However, whether E2 has a positive or a negative effect on glucose homeostasis is still a matter of debate. Many authors consider that E2 at physiological levels is involved in the maintainance of normal insulin sensitivity, but outside the physiological range, E2 may promote insulin resistance and diabetes.[13,14] The notion that high E2 concentration is detrimental to blood glucose homeostasis dates back to 1960s when it was reported that some women taking high-estrogen oral contraceptives developed insulin resistance.[15,16] On the other hand, women with low serum levels of estrogens, as for example during menopause, are at greater risk of developing type 2 diabetes.[13,15] Estrogen replacement in postmenopausal women, depending on dose and duration of treatment, is associated with an improvement of insulin sensitivity and a reduction of blood glucose, lipid, cholesterol levels and body fat.[17-19]

Aromatase knockout (ARKO) and ERα knockout (ERKO) mice provided the first evidence of the contribution of estrogen/ERα signaling to glucose metabolism by demonstrating that both animal models showed glucose intolerance and insulin resistance.[20,21] Analogous findings were also found in humans; patients with aromatase deficiency suffered from impairment of glucose metabolism and presented insulin resistance.[22] Regarding ERα function, only one case has been described in the literature. It concerns a male with estrogen deficiency due to an inactivating mutation of the ERα gene. The man showed glucose intolerance in association with high serum E2, estrone, FSH and LH levels.[22,23] Moreover, genetic polymorphism of the ERα gene in humans has been associated with type 2 diabetes and the metabolic syndrome.[24]

Although up to now evidence has pointed to ERα as the main mediator of the regulatory effect of E2 on glucose homeostasis, both ERα and ERβ have been associated with the control of energy balance. The following is a brief overview of the action of both receptors in different tissues.

The disruption of ERα in the ventromedial nucleus of the hypothalamus leads to weight gain, increased visceral adiposity, hyperphagia, hyperglycaemia and impaired energy expenditure in female mice.[25] Moreover, ERβ has been shown to have anorectic effects mediated via the central nervous system.[26]

In the liver, ERα is involved in the modulation of insulin sensitivity, proof of this being the fact that

ERKO mice develop severe hepatic insulin resistance with an associated decreased glucose uptake in skeletal muscle.[27]

GLUT 4 is the major insulin-stimulated glucose transporter and constitutes the main rate-limiting step in insulin-stimulated glucose transport both in muscle and adipose tissue. In 2006, Barros et al reported that both receptors (ERα and ERβ) can modulate GLUT4 expression in skeletal muscles of mice.[28]

Regarding adipose tissue, it has long been proposed that estrogens can control the distribution of body fat and metabolism, and this action is thought to be mediated by ERα and ERβ. [29-32] The absence of ERα produces adipocyte hyperplasia and hypertrophy in white adipose tissue and is followed by insulin resistance and glucose intolerance;[21,29] meanwhile, ovariectomy of ERKO mice improves insulin resistance.[31] In humans, both receptors may play an important role in fat metabolism. The ratio ERα/ERβ seems to be associated with obesity as well as with prolactin serum level and production of leptin in the omental adipose tissue in women.[33]

The role of estrogen receptors in the endocrine pancreas is gradually being elucidated. We have recently demonstrated that E2 increases insulin mRNA levels and insulin biosynthesis, incrementing insulin content and insulin release in an ERα dependent manner.[34] Besides the role that ERα plays in the regulation of pancreatic insulin content, it can partly mediate the antiapoptotic effect that E2 has in pancreatic β-cells after streptozotocin treatment.[35] Recently, we have reported that ERβ has a rapid insulinotropic action that involves the atrial natriuretic peptide receptor.[36]

3. COULD ENVIRONMENTAL ESTROGENS DISRUPT GLUCOSE HOMEOSTASIS? THE CASE OF BPA

Growing evidence accumulated over the last decade supports the hypothesis that many chemicals in the environmental can interfere with complex endocrine signaling mechanisms and cause adverse consequences. These chemicals are collectively termed endocrine disrupting chemicals (EDCs), endocrine disruptors or environmental estrogens. They have been defined by the Environmental Protection Agency (EPA) as "an exogenous agent that interferes with the production, release, transport, metabolism, binding, action, or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes".[37] A large number of EDCs act by mimicking the action of E2 [38] and, based on this evidence, we hypothesized that exposure to an exogenous chemical acting as the natural hormone but in an inappropriate concentration and during an improper time window may affect multiple organ system development and function including control of energy balance and glucose homeostasis. Among the various EDCs we selected to focus our attention on the effects of bisphenol-A (BPA).

BPA from bench to human exposure

Bisphenol-A (BPA) was first synthesized by Dianin in 1891 and was reported to be a synthetic estrogen in the 1930s.[39] At the same time, diethylstilbestrol (DES) was also tested and, due to its strong estrogenic activity, BPA essentially took a backseat. In the 1950s, BPA was rediscovered as a compound that could be polymerized to make polycarbonate plastic, and from that moment until the present day it has been commonly used in the plastics industry. BPA is one of the highest volume chemicals produced worldwide, with over 6 billion pounds produced each year and over 100 tons released into the atmosphere yearly.[40] It is used as the base compound in the manufacture of polycarbonate plastic and the resin lining of food and beverage cans, as well as an additive in other widely used plastics such as polynil chloride and polyethylene terephthalate. It is present not only in food and beverage containers but also in some dental material.[41] Numerous studies have found that BPA can leach from polycarbonate containers; heat and either acidic or basic conditions accelerate hydrolysis of the ester bond linking BPA monomers, leading to release of BPA and thus concomitant potential human exposure.[42,43] Indeed, the potential for BPA exposure was demonstrated when BPA was detected in 95% of the urine samples in the USA.[44] Its concentration in human serum ranges from 0.2 to 1.6 ng/ml (0.88-7.0 nM).[45,46] Moreover, it has been detected in amniotic fluid, neonatal blood, placenta, cord blood and human breast milk.[43]

Concerning the potential risk of BPA, the lowest-observable adverse effect level (LOAEL) was set in the 1980s at 50 mg/kgBW/day and the Environmental Protection Agency (EPA) calculated a "reference dose" or safe dose of 50 μg/ kgBW/day. However, since that time, abundant scientific evidence has demonstrated that BPA can interfere with the endocrine signaling pathways at doses below the calculated safe dose, particularly after exposure during fetal, neonatal or perinatal periods, but also in adulthood. A review by Richter and colleagues provides a comprehensive account of the findings from *in vivo* studies of BPA exposure.[47]

Tissue-specific analysis of BPA. Effects on insulin sensitivity by using animal models

- Effects on peripheral tissues:

Recently, we have demonstrated that the administration of BPA in adult male mice provokes hyperinsulinemia and mild insulin resistance.[48] This phenomenon, as is described below, is at least partly due to a direct effect of BPA on the endocrine pancreas, although the possibility exists that BPA may also have direct effects on peripheral tissues. Indeed, unpublished results from our group have shown that BPA can also alter insulin signaling in skeletal muscle and adipose tissue.

There are also some studies documenting the hepatic effects of BPA. It has been proposed that BPA can cause abnormalities in the liver of rats and mice, since administration of BPA induces oxidative stress by decreasing antioxidant enzymes both at low (0.2-20 μg/kg)[49] and high doses (25-50 mg/kg).[50]

Regarding adipose tissue and energy balance, a significant reduction in body weight and food intake was reported when ovariectomized adult female rats were treated with high doses of BPA,[51] while other studies did not find alteration in body weight, fat depots or trygliceride levels at low doses (33-333 μg/kg).[52] On the other hand, Sakurai et al reported that BPA can affect glucose transport in adipocytes. They have demonstrated that in the presence of BPA there is an increase of basal and insulin-stimulated glucose transport due to an increased amount of GLUT4.[53] Others have shown that BPA stimulates adipogenesis in 3T3-L1 adipocytes.[54,55] Moreover, exposure of the fetus to low doses (25 μg/kg/day) of BPA in rats resulted in high birth weight.[56,57] Interestingly, it has been reported that BPA at 1 and 10 nM concentrations inhibits adiponectin release, an important adipokine that protects humans from the metabolic syndrome.[58]

- Effects on the endocrine pancreas:

Generally, estradiol action is considered to occur in the nucleus and involves the direct participation of estrogen receptors (ERα and ERβ) as transcription factors. In addition, it has been shown that there are many other alternative pathways that can mediate estradiol action.[10,59] It is thought that ERα and ERβ from outside the nucleus, in the cytosol and the plasma membrane, are able to activate other signaling cascades. Other novel membrane ERs have also been described; these novel receptors may be isoforms of ERs obtained by alternative splicing or completely new proteins encoded by different genes.[60] Moreover, estradiol can act by binding directly to other neurotransmitter receptors and to ion channels.[61,62] Most frequently, these alternative pathways occur within seconds or minutes after addition of estradiol and for that reason they have been named rapid estrogen effects.

a. Rapid effects of BPA on pancreatic α-cells

Diabetes mellitus denotes malfunction of the β-cell and decreased insulin secretion. However, we should keep in mind that α-cells also play an important role in the regulation of glycaemia and nutrient homeostasis and that diabetes is associated with disorders in the levels of both insulin and glucagon.[63,64] Pancreatic α-cells represent 5-10% of the cell population of the islets of Langerhans and coexist along with insulin-secreting β-cells, δ-cells and PP cells. They secrete glucagon in response to low glucose concentration in a Ca^{2+} dependent manner. Glucagon enhances the synthesis and mobilization of glucose in the liver and, in addition, it has many extrahepatic effects, such as the increase of lipolysis in adipose tissue, a positive ionotropic effect in the heart, a role in the satiety control in the central nervous system and the regulation of the glomerular filtration rate.[64] Little is known about the stimulus-secretion coupling in α-cells but it seems to contain a specific

set of ion channels, including a voltage-dependent Na^+ channel, responsible for their electrical activity;[65-67] the intracellular calcium ion $[Ca^{2+}]_i$ oscillating at low glucose concentration.[68,69] Because of the calcium influx, the exocytotic machinery is initiated and glucagon is released.[70,71] When the extracellular glucose concentration increases to the level required for insulin to be released, the frequency of $[Ca^{2+}]_i$ oscillations diminishes and, as a result, glucagon release decreases.[69,72]

In previous studies we have demonstrated that the xenoestrogen BPA at a concentration of 1 nM suppresses low glucose-induced intracellular calcium ion oscillations. This action is characterized by rapid onset and is initiated at the level of plasma membrane. The intracellular pathway triggered by the binding of BPA involves a pertussis toxin sensitive G-protein, nitric oxide synthase, guanylate cyclase and PKG.[73] These results suggest that BPA may alter both glucose and lipid metabolism.

b. Rapid effects of BPA on pancreatic β-cells

As with α-cells, β-cells are also excitable cells, their ion channels generating an oscillatory electrical activity that causes an intracellular oscillatory $[Ca^{2+}]_i$ pattern. Remarkably, this oscillatory pattern triggers a pulsatile insulin secretion.

We have previously reported that estradiol provokes the closure of the K_{ATP} channel of pancreatic β-cells in a rapid manner. The maximum inhibition of the channel is reached 3 to 7 minutes after estradiol application and the effect is transient returning to normal levels 30 minutes later. As a consequence, there is an increased frequency $[Ca^{2+}]$ oscillations and a enhanced insulin secretion (rapid insulinotropic effect) that occurs when estradiol is applied along with a stimulatory glucose concentration. It has also been demonstrated that the fast modulation of insulin secretion by estradiol is not a genomic effect, since neither actinomycin-D nor cycloheximide prevent it. Mechanistically we know that ERβ but not ERα mediates a rapid estradiol effect on β-cells. We have proposed that, in synergy with glucose, when estradiol binds to ERβ, the guanylate cyclase A receptor is activated through a yet unknown mechanism. As a consequence, K_{ATP} channel activity decreases in a cGMP/PKG-dependent manner, which finally potentiates an enhanced insulin secretion. These experiments have been done *ex vivo*.[36,74]

We have also demonstrated that the rapid insulinotropic effect of estradiol also occurs *in vivo.* Thus, the injection of 10 μg/kg of estradiol provoked a decrease of glycaemia 30 minutes after the administration, in parallel to an increase of plasma insulin levels.[48]

As regards BPA effect, it has been shown by experiments performed *ex vivo* that BPA rapidly enhances the frequency of glucose-induced $[Ca^{2+}]$ oscillations in pancreatic β-cells as does estradiol. This effect is triggered by remarkably low concentrations of BPA within the nanomolar range; 0.1 nM of BPA is enough to elicit a significant effect.[75] The mechanism that mediates this effect is still unresolved, but unpublished experiments by our group indicate that it is most probably the same as that of estradiol.

In vivo, BPA rapidly change glycaemia by inducing a hypersecretion of insulin. As happens with estradiol, 30 min after the administration of 10 μg/kg of BPA there is an increase in plasma insulin levels.[48]

An additional rapid effect of BPA and E2 on isolated islet cells has also been observed; they increase the activation of the ubiquitous transcription factor cAMP response element binding protein (CREB) 5 min after stimulation at doses as low as 1 nM.[76] This effect may be of great importance for the β-cell physiology, since CREB activation induces insulin gene expression[77] and is implicated in β-cell survival.[78]

c. Long-term effects of BPA on pancreatic β-cells

In order to study the long-term action of BPA on the physiology of β-cells, we treated male mice with a daily dose of either 100 μg/kg BPA or E2 for 4 days. After the treatment, we observed a higher insulin content in β-cells compared to vehicle-treated mice. This effect followed an inverted U-dose response and the increase was already detected at day 2. Moreover, we demonstrated that this increase in insulin content leads to an enhanced insulin secretion. The β-cells from animals treated with BPA not only had more insulin but they also released more insulin when they were stimulated with high glucose concentrations.[48]

This result is consistent with the potentiation of insulin release observed *in vitro* in response to glucose after incubating rat islets with BPA for 24 h.[79]

At plasma level, the 4 days treatment with BPA generates a post-prandial hyperinsulinaemia. When a glucose tolerance test was performed in these mice under fasting conditions, an impaired glucose tolerance was observed, indicating that these animals were insulin resistant, which was confirmed by an insulin tolerance test; in response to a challenge of insulin, the hypoglycaemic response of BPA-treated mice was lower compared to vehicle treated mice.[48]

Whether insulin resistance precedes hyperinsulinaemia or hyperinsulinaemia precedes insulin resistance in the development of type 2 diabetes is controversial. The only clear conclusion is that they occur in parallel.[80] We cannot rule out the possibility that peripheral insulin resistance contributes to the hyperinsulinaemia detected in BPA-treated mice. Nevertheless, we have demonstrated that BPA increases insulin content and insulin release when the islets of Langerhans are cultured in the presence of BPA, suggesting that BPA has a direct effect on the islets. BPA treatment did not have any effect on β-cell survival or β-cell mass, but it did have an impact on the insulin gene transcription, provoking an upregulation of the gene in an ERα-dependent manner.[34]

Epidemiological evidence for the link between BPA and metabolic disorders

Studies in animal models and *in vitro* studies provide clear evidence of the potential adverse effects of BPA, but the critical questions is: can we extrapolate these findings to the human disease process? This is a very difficult question to answer but, in fact, there is mounting epidemiological evidence that passive absorption of EDCs from the environment may well be related to the alarming rate of diabetes and obesity.

There is also evidence of persistent organic pollutants (POPs) exposure. Thus, white adipose tissue represents a reservoir of lipophilic environmental pollutants, especially those resistant to biological and chemical degradation, the so-called POPs. Some of these POPs, like dioxins, furans, polychlorinated biphenyls (PCBs) or organochlorine pesticides, have been strongly associated with diabetes and most of the components of the metabolic syndrome in several cross-sectional studies.[81,82] This association is partly based on several epidemiological studies of serum γ-glutamyltransferase (γGT). Serum γGT activity may reflect amounts of glutathione conjugates formed during the metabolism of xenobiotics and it has been proposed that the association of serum γGT with type 2 diabetes reflects exposure to POPs and that POPs interact with obesity to cause type 2 diabetes.[83]

Regarding the potential detrimental action of BPA, a major epidemiological study has recently been published in which a significant relationship between BPA concentration in urine and type 2 diabetes, cardiovascular disease and liver enzyme abnormalities is established.[84,85] To our knowledge, this is the first confirmation of adverse effects of BPA on humans namely, effects on insulin homeostasis and liver enzymes previously reported in animal models.

CONCLUSIONS

The data reviewed in this communication show that environmental estrogens, in particular BPA, are most likely implicated in the exacerbation and acceleration of type 2 diabetes development. In adult humans, epidemiological evidence points to BPA as an important risk factor for type 2 diabetes. In addition, there are casual links between BPA exposure and insulin resistance: alterations in insulin biosynthesis and secretion by β-cells of adult male mice and decrease of adiponectin in human adipocytes. Insulin resistance and decrease of adiponectin are highly likely to contribute to the development of type 2 diabetes, especially in subjects with a genetic susceptibility to β-cell failure.

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