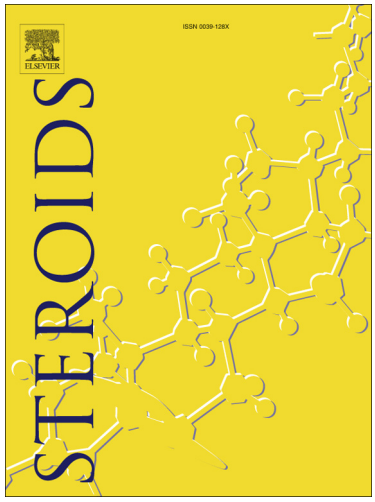

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Effects of Bisphenol A on ion channels: experimental evidence and molecular mechanisms

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Abstract

Bisphenol A (BPA) is an endocrine-disrupting chemical (EDC) produced in huge quantities in the manufacture of polycarbonate plastics and epoxy resins. It is present in most humans in developed countries, acting as a xenoestrogen and it is considered an environmental risk factor associated to several diseases. Among the whole array of identified mechanisms by which BPA can interfere with physiological processes in living organisms, changes on ion channel activity is one of the most poorly understood. There is still little evidence about BPA regulation of ion channel expression and function. However, this information is key to understand how BPA disrupts excitable and non-excitable cells, including neurons, endocrine cells and muscle cells. This report is the result of a comprehensive literature review on the effects of BPA on ion channels. We conclude that there is evidence to say that these important molecules may be key end-points for EDCs acting as xenoestrogens. However, more research on channel-mediated BPA effects is needed. Particularly, mechanistic studies to unravel the pathophysiological actions of BPA on ion channels at environmentally relevant doses.

Abbreviations

AMPA, α-Amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; BPA, Bisphenol A; ER, estrogen receptor; CFTR, Cystic fibrosis transmembrane conductance regulator; $GABA_A$, Gamma-aminobutyric acid type A; EDCs, Endocrine-disrupting chemicals; GPR30 or GPER, G-protein coupled receptor 30 or G-protein coupled estrogen receptor; HEK, Human embryonic kidney cells; I_{CRAC}, Ca^{2+} release-activated currents. NMDA, *N*-methyl-d-aspartate; *o*, *p'*-DDT, 4-Octylphenol p-nonylphenol dichlorodiphenyltrichloroethane; Orai1, Calcium release-activated calcium channel protein 1; RyR, Ryanodine receptors; tbBPA, TetrabromoBPA; TTX, Tetrodotoxin; TRP, Transient receptor potential.

Keywords

Bisphenol A, ion channel, endocrine disruptor, environmental doses, 17 β-Estradiol, pancreatic β-cells.

Highlights

► The effects of Bisphenol A (BPA) on ion channels are reviewed.

► The range of BPA experimental concentrations comprises from environmentally relevant doses of BPA to high doses of BPA, unusually found in humans.

► The function of a plethora of ion channels are affected by BPA.

► More systematic studies are needed to unravel the health related actions of BPA on ion channels at environmentally relevant doses.

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1. Introduction

During the last 20 years, a growing number of scientific studies have largely strengthened the evidence for endocrine health-related actions of endocrine-disrupting chemicals (EDCs). These currently widespread substances are defined by the Endocrine Society as: "an exogenous chemical, or mixture of chemicals, that interfere with any aspect of hormone action" [1]. These compounds can persist in the environment and, once they gain access to the human body, they can alter the hormonal milieu and affect many target tissues and physiological functions, being the translational evidence strongest for obesity and diabetes, female and male reproduction, hormone-sensitive cancers in females, prostate, thyroid and neurodevelopment and neuroendocrine systems [2]. Given that general population is exposed to a complex mixture of environmental chemicals, many of which are known EDCs, the potential human health risk is substantial and far beyond the relatively modest effect that each of these chemicals may have individually.

Although there may be hundreds or more environmental chemicals with EDC activity, Bisphenol A (BPA) is one of the most commonly studied and one of the longest known, since it was discovered to be estrogenic in 1936 [3]. BPA is produced in higher amounts than any other chemical, with 15 billion pounds yielded in 2013 [4]. BPA is the building block of polycarbonate plastic, often used for food and beverage storage and a component of epoxy resins which are used to line food and beverage containers. Thus, food intake and drink are generally considered the major exposure route. The presence of BPA in our environment is ubiquitous and 93% of U.S. population has a measurable amount of BPA in their urine [5]. The mean BPA levels in human serum reported ranged from 4.3 ng/ml in children, 2.8 ng/ml in adolescents and 2.3-2.4 ng/ml in adults, although measurements of bioactive or free BPA in human serum is controversial at present. In addition, BPA has been detected in human amniotic fluid, neonatal blood, placenta, cord blood and human breast milk and interestingly BPA is fat-soluble and thus can accumulate in fatty tissues [6]. BPA is rapidly metabolized to nonbioactive forms and has a short half-life of approximately 4–5 hours in adult humans, with lower metabolic rates in the fetus and infants [7-9]. Currently, the US Environmental Protection Agency (EPA) safety level of BPA is set at 50 μg/kg/day, whereas the European Food Safety Authority's temporary tolerable daily intake was recently lowered to 4 μg/kg/day. Risk assessments for BPA have considered that oral exposure

is the main and almost exclusive exposure source, however, increasing evidence shows that additional routes like transdermal one, constitutes an important non-food source of BPA contamination [10, 11].

Estrogenic compounds like BPA are able to mimic the effect of the natural hormone17β-estradiol, at the same doses, and through common signaling pathways [12, 13]. Estrogens, like other sex hormones, regulate the functioning of tissues in addition to those involved in reproduction in adults. This hormone plays an important role in the physiology of a variety of tissues including the brain, the reproductive and the cardiovascular system, directly modulating the function of many cells through activation of steroid receptive molecules, mainly the estrogen receptors α and β. In addition, some cells also express the G-protein coupled receptor for 17β-estradiol, named GPR30 or GPER (G-protein coupled estrogen receptor) [14, 15].

Many of the actions of common intracellular signaling pathways, including those initiated by estrogens, are mediated by ion channels.Given the central role of ion channels in the physiology of the cell, it is not surprising that defects in the pathway that regulates channel function may contribute to the development of health disorders. Although it is well established that 17β-estradiol modulates several ion channels, both through classical genomic as well as rapid non-genomic pathways [16-18], the action of BPA at environmentally relevant doses it is still poorly investigated.

The number of publications reporting the effect of BPA on ion channels in different cell types is still very scarce. It is of note that the doses used in many of these studies are usually at the micromolar range, which is a concentration not usually found in animal organisms. In these conditions BPA may act through mechanisms other than activation of estrogen receptors, i.e: the direct docking of BPA moieties to the ion channel pore. These studies, although important, do not completely clarify the mechanisms through which the exposure to environmentally relevant doses of BPA alter the physiology of humans, as epidemiological studies suggest [19]. Strikingly, in most of these studies using high BPA concentrations, the dose response curve has not been completed to smaller doses. This is an important issue given the accepted fact that dose response are non-monotonic for many hormones and EDCs and low-dose effects seem to be the most relevant for BPA environmental disruption [20].

The aim of this paper is to review the effects of BPA on ion channels described in the literature. We believe that it will encourage a more detailed study of the actions of BPA on ion channels at environmentally relevant doses and hence, bringing mechanistic insight into the suggested association between BPA exposure and health disorders. The table and figure below comprises a comprehensive summary of the effects of BPA on ion channels (table 1 and figure 1). The upper limit of environmentally relevant dose in the present review has been established as a maximal BPA administration of 500 μg/Kg/day, which according to some pharmacokinetic studies, leads to a ratio of serum conjugated to unconjugated BPA within the range reported in human biomonitoring studies [21, 22]. Similarly, regarding BPA applied directly to a tissue/cell preparation, we have considered as physiologically relevant doses those in the nanomolar range (up to 100 nM). This "low-dose" cut-off has been defined by the Chapel Hill expert panel and has been widely adopted [23].

In the sections below, different ion channel types are considered in relation to the reported actions of BPA on them. Furthermore, in order to illustrate the extent to which the interaction between BPA and ion channels may derive in actual human health disorders, we have included a final section about ion channel-mediated actions of BPA in pancreatic β-cells. Two reasons validate this choice: the first one, that much work about ion channel regulation by estrogens and BPA has been developed in this cell type. The second reason is the strong translational evidence already accumulated for BPA exposure as a risk factor for obesity and diabetes [2, 13, 19].

2. BPA actions on Na^+ channels

Na^+ channels play a central role transmitting depolarizing impulses rapidly throughout cells and cell networks, thereby enabling coordination of higher processes ranging from locomotion to cognition.

Two electrophysiological studies have reported that high doses of BPA (micromolar range) inhibits voltage-gated Na^+ channels (both TTX-sensitive and TTX-resistant channels) by direct binding of the BPA molecule to the channel [24, 25]. This indicates that BPA might have potential toxicological effects on the nervous system. In contrast, Belcher and coworkers have reported an increase in the mRNA expression of voltage-

gated Na^+ channel, Nav2.3 and Navβ4 (β subunit of Na^+ channels) in the left ventricle of female mice with low doses of BPA (1 nM) that could account for altered cardiovascular functioning reported in epidemiological studies [2, 26].

3. BPA actions on K^+ channels

Voltage- and Ca^{2+}-gated K^+ channels (BK, maxiK or $K_{Ca}1.1$) are essential regulators of several key physiological processes including smooth muscle tone and neuronal excitability. Two studies have characterized the effect of high doses of BPA (1-100 μM) in this channel type in coronary smooth cells [27] and in transfected cells [28], revealing a direct channel activation by BPA via α and β1 subunits, similar to that of estradiol [18, 29]. These data indicate that the increased activity of Maxi-K channels induced by BPA may represent a basis for potential toxicological effects.

Another study by Ito and coworkers with high doses of BPA (100 μM) has reported a direct block of K_{cAMP} (a K^+ slow rectifier voltage-gated channel, modulated by cAMP) and of K_{Ca} (a Ca^{2+}-gated K^+ channel of intermediate conductance) in human airway epithelial cells, which interrupts CFTR-mediated Cl^- secretion. This effect was caused independently of estrogen receptors, suggesting a binding site for BPA at high doses on both K^+ channels [30].

Belcher and coworkers [26] have reported a decrease in the mRNA expression of several K^+ channels: $K_v1.1$ and $K_v1.6$ (shaker-related voltage-gated K^+ channels), $K_{ir}4.1$ (an inwardly rectifying K^+ channel) and Mirp3 (a β subunit of voltage-gated K^+ channels) in the left ventricle of male mice with low doses (1nM) of BPA that could account, as stated above, for the effect on Na^+ channels on cardiovascular health.

4. BPA actions on Ca^{2+} channels

Ca^{2+} ions play crucial roles in regulating a variety of cellular functions in many different cell types. Several works have addressed the effect of BPA on different types of Ca^{2+} channels, including voltage-gated Ca^{2+} channels, ryanodine receptors (RyR), transient receptor potential (TRP) channels and Orai1 (Calcium release-activated calcium channel protein 1). Glutamate ionotropic receptors permeable to Ca^{2+} are commented in a section below.

Ca^{2+} channels in many different cell types activate with membrane depolarization and mediate Ca^{2+} influx in response to action potentials and subthreshold depolarizing signals. On the basis of their voltage dependence of activation, the voltage-gated Ca^{2+} channels fall into two broad groups: the low (T-type) and the high (L-, N-, P/Q- and R-type) threshold activated channels.

A recent study reported an increase in mRNA expression of T-type voltage-gated Ca^{2+} channels induced by low doses of BPA in male mice reproductive system and suggested that estrogen receptor signaling was necessary for this effect [31]. This action of BPA could account for the effects on reproductive function observed in epidemiological studies [2]. However, in human embryonic kidney 293 (HEK) cells expressing T-type Ca^{2+} channels, acute application of BPA at different doses (0.1 nM-100 μM) inhibited the Ca^{2+} current through this channel. Although high doses were required for inhibition of $Ca_v3.1/3.3$ subtypes, lower doses (within the nanomolar range) were sufficient to inhibit $Ca_v3.2$ subtype [32] in concentrations in the range of those detected in human fluids and could be relevant for evaluation of health effects of BPA.

An electrophysiological study has reported that Ca^{2+} currents through native L-, N-, P/Q-, T-type Ca^{2+} channels in rat endocrine GH3 cells, mouse dorsal root ganglion neurons or cardiac myocytes, and recombinant human R-type Ca^{2+} channels expressed in HEK cells, were rapidly and reversibly inhibited by high doses of BPA with similar potency (EC_{50} values: 26–35 μM), acting directly on the channel protein [33]. In another study, the mRNA expression of an auxiliary subunit of the high threshold voltage-gated Ca^{2+} channel, Cacnα2δ2, was decreased in the left ventricle of male mice with low doses of BPA (1 nM) [26].

Another study with high doses of BPA (25-150 μM) proposed a model for non-genomic action of BPA in PC12 cells, demonstrating the participation of G-protein, cyclic AMP/PKA, N-type Ca^{2+} channels, and ryanodine-sensitive Ca^{2+} stores in BPA-induced increase in dopamine release [34].

Ryanodine receptors have been as well investigated in two other studies. A work using tetrabromoBPA (tbBPA), which is a commonly used brominated flame retardant to reduce the flammability of several products, reported an inhibition of RyRs in Sertoli

cells that may cause endocrine disruption and Ca^{2+} dysregulation in cells involved in spermatogenesis, although the doses of tbBPA used in this case were high (5-60 μM) [35]. Later, Gao and coworkers have reported that exposure to low doses of BPA (1 nM) increased both the production of cAMP and the phosphorylation of RyRs by PKA through ERβ in cardiac ventricular myocytes. Phosphorylation of the RyR increases RyR open probability and Ca^{2+} release from sarcoplasmic reticulum. These results provide a mechanistic insight into BPA's rapid proarrhythmic actions in female cardiac myocytes and contribute to assess the potential cardiac toxicity of BPA exposure [36].

To date, just one study has reported an effect of BPA on TRPs [37]. High doses of BPA (5000-50000 μg/kg/day) were given to pregnant mice from gestation day 6.5 to 16.5. mRNA expression and protein levels decreased for both TRPV5 and TRPV6 channels in the kidney and duodenum, suggesting that BPA regulates the expression of genes associated with Ca^{2+} transport in pregnant mice, which may result in decreased serum Ca^{2+} levels.

Orai1 is a Ca^{2+} selective ion channel activated upon depletion of internal Ca^{2+} stores and it mediates the Ca^{2+} release-activated currents (I_{CRAC}). A recent study has reported an increase in Orai1 protein levels induced by low doses of BPA (1-10 nM) in prostate cancer cells, leading to an amplification of the store operated Ca^{2+} entry and stimulating cancer cell migration [38]. These results provide novel insights into the molecular mechanisms involved in the effects of an environmental factor on cancer cells.

5. BPA actions on Cl^- channels and aquaporins

Cl^- channels are a functionally and structurally diverse group of anion selective channels involved in processes that include regulation of a broad array of features, like excitability in neurons, skeletal, cardiac and smooth muscle, cell volume regulation, transepithelial salt transport, acidification of internal and extracellular compartments, cell cycle and apoptosis. Cl^- channels can be classified into voltage-sensitive, calcium-activated, high (maxi) conductance channels, the cystic fibrosis transmembrane conductance regulator (CFTR) and volume regulated channels. Aquaporins are integral membrane proteins that operate as water transfer channels, widely conserved in bacteria, plants, and animals [39, 40].

To date, only two studies have reported actions of environmentally relevant doses of BPA on Cl^- channels and aquaporins. Administration of BPA (0.1-10-1000 ppm) to male mice in a trans-generational protocol increased the expression of CFTR channels and aquaporins in the reproductive system, producing a detrimental effect on the reabsorptive activity of epididymis that could affect male fertility [41]. In another study, low doses of BPA (1 nM) decreased the mRNA expression of the Cl^- voltage-gated channel CLC1 in the left ventricle of male mice; conversely, aquaporin 7 expression was increased [26]. These results may account for cardiovascular health disorders, as stated above.

6. Ionotropic glutamate receptors.

Ionotropic glutamate receptors mediate fast excitatory synaptic transmission in the central nervous system and regulate a broad spectrum of processes in the brain, spinal cord, retina, and peripheral nervous system. Glutamate receptors play important roles in numerous neurological diseases and have attracted intense scrutiny. Several studies have investigated the effects of BPA on glutamate-gated ionotropic NMDA (*N*-methyl-D-aspartate) and AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) receptors [42, 43].

The first study by Sato and coworkers reported an increased expression of NMDA GluN1 receptors in hippocampal slice cultures upon 1 nM BPA incubation for one day, which was not mediated by estrogen receptors. This effect exacerbated the neuronal damage caused by glutamate and upregulated the spine density of the apical portion of dendrites [44]. Later, in 2010, two papers reported a decrease of NMDA receptors in mice hippocampus. Prenatal and postnatal exposure to BPA at low doses (100-500 μg/Kg/day) decreased NMDA (not subtype specified) and dopamine receptors, and induced anxiolytic behaviors as well as cognitive deficits [45]. At high doses (50-50000 μg/Kg/day) BPA inhibited the expression of NMDA receptors GluN1, GluN2A and GluN2B and ERβ, impairing learning and memory [46] That same year, two other papers reported opposite effects of low doses of BPA on NMDA receptors in rat hippocampus: a decrease in the expression of NMDA receptors [47] and an increase in NMDA receptors GluN2B mediated by ERβ phosphorylation promoting dynamic changes in hippocampal dendritic morphology [48] A further paper by Xu and coworkers reported again an increase in the expression of NMDA receptors GluN1and

GluN2B mediated by non-genomic ER phosphorylation of receptors in rat hippocampus, thus enhancing passive avoidance memory [49]. Two other papers by the same group reported a decreased expression of NMDA and AMPA receptors in mice hippocampus using a transgenerational protocol of BPA administration (400-40000 μg/Kg/day) inhibiting synaptogenesis and sex-specific effects in memory and synaptic structure [50, 51]. It is remarkable that most of the effects of BPA on ionotropic glutamate receptors by Xu and coworkers were observed at doses relevant to human exposure.

7. BPA actions on nicotinic receptors

Nicotinic receptors are activated by the endogenous neurotransmitter acetylcholine and they are expressed by both neuronal and non-neuronal cells throughout the body. These receptors form ion channels permeable to cations, regulating physiological processes ranging from maintenance of metabolic tone, to control of inflammatory processes or their widely studied influence over inhibitory and excitatory transmissions in the nervous system.

One of the first studies investigating the action on BPA was performed on neuronal α3β4 and α4β2 nicotinic receptors expressed in *Xenopus* oocytes using electrophysiological techniques [52]. High concentrations of BPA (0.1-100 μM) were acutely applied to these ion channels, producing a direct block that was independent of estrogen receptors. For this reason, it will here be considered a pharmacologic study, not representing the actual actions of BPA in human fluids, which hardly ever may achieve these high concentrations.

8. BPA actions on GABA receptors

GABA (gamma-aminobutyric acid) is an inhibitory neurotransmitter very widely distributed in brain neurons. GABA contributes to motor control, vision, and many other cortical functions. $GABA_A$ receptors are channel proteins allowing negatively charged Cl^- ions to enter the neuron, thus reducing its excitability.

The first study reporting an action of BPA on an ion channel was performed on $GABA_A$ receptors expressed in *Xenopus* oocytes [53]. BPA has dual actions on $GABA_A$ channels, depending on the concentration of BPA used: 0.1 mM BPA potentiates, and 1

mM BPA inhibits those currents generated by 10 μM GABA. It has to be noted, however, that these are extremely high concentrations of BPA and that the effect was induced by direct binding of BPA to $GABA_A$ channels, independently of estrogenic actions. Later, in 2007, another paper reported multiple effects of BPA on $GABA_A$ receptors in hippocampal neurons. Doses of BPA were high (3-300 μM) and the direct effect of BPA depended on the dose of GABA used to activate the channel. A high concentration of BPA potentiated the action of GABA at low doses and inhibited the effect of GABA at high doses [54].

A study of perinatal administration of BPA at low doses reported an inhibition of $GABA_A$ currents in neurons of the amygdala, causing GABAergic disinhibition and dopaminergic enhancement, leading to an abnormal cortical-amygdala synaptic transmission and plasticity, which may be responsible for the hyperactivity and attention-deficit in BPA-exposed rats [55]. Finally, a recent paper has reported sex-specific effects of long-term exposure to BPA on $GABA_{A2}$ receptor levels in the hippocampus, increasing it in females and decreasing it in males. These results, together with an altered behavioral assay, suggest that long-term exposure to BPA affects anxiety- and depression-like behaviors in adult mice mediated by changes in the levels of $GABA_{A2}$ receptor and ERβ proteins of hippocampus [56].

9. Ion channel regulation by estrogens and BPA in pancreatic β-cells

Pancreatic β-cells are electrically excitable cells. They have a central role in glucose homeostasis and represent the most abundant cell type in the endocrine pancreas [57-59]. The main task of pancreatic β-cells is the biosynthesis and release of insulin in response to an increase of plasma glucose concentration. Briefly, at low glucose concentrations, K_{ATP} channels are mostly in the open state and the cell membrane resting potential remains around -70mV. However, when glucose levels increase, ATP/ADP ratio raises, leading to the closure of K_{ATP} channels. As a consequence, membrane potential depolarizes up to -40mV, opening voltage-dependent Ca^{2+} channels and increasing intracellular calcium [60, 61]. $[Ca^{2+}]_i$ and cyclic AMP oscillatory pattern originate, triggering a pulsatile insulin secretion to counteract the rise in glucose plasma concentration [62-64]. As in neurons, estrogens and EDCs can modulate ion

channels in pancreatic β-cells. Here we give a summary of the thoroughly described effects of BPA on insulin-secreting cells.

A key ion channel in pancreatic β-cells is the K_{ATP} channel. It plays a crucial role in stimulus-secretion coupling. It is an octameric complex composed by four Kir6.2 and four sulfonylurea receptor SUR1 that comprises the regulatory subunit[14, 60, 65]. In physiological conditions ATP/ADP ratio, long-chain acyl CoA esters (LC-CoAs) and phosphatidylinositol-4,5- bisphosphate, among other intracellular factors, can modulate its activity [66]. However, it has been demonstrated that other compounds like estrogens can regulate its function in a non-genomic manner. Previous studies in mice demonstrated that physiological concentrations of 17β-estradiol (100 pM-1 nM), in synergy with glucose, decreased K_{ATP} channel activity via cGMP production, thus increasing $[Ca^{2+}]_i$ oscillations in a concentration-dependent manner. It was demonstrated that the receptor involved in this process was located ~~i~~outside the nucleus, and it waswas proposed to be different to the classical cytosolic estrogen receptors ERα and ERβ [67-69]. This non-classical membrane estrogen receptor could be GPER which is also expressed in β-cells [70].In 2009, however, Soriano et al. demonstrated that ERβ, but not ERα, rapidly modulated K_{ATP} channels. 17β-estradiol (1 nM) via ERβ, decreased K_{ATP} channel activity in a cGMP dependent manner. This mechanism potentiated calcium signalling and insulin secretion [71].

Additional effects of SUR1 subunit, besides regulating electrical activity, have been proposed. 17β-estradiol directly interacts with K_{ATP} channels modulating β-cell apoptosis under specific involvement of SUR1 in an age-dependent manner. Authors have shown that 100 μM 17β-estradiol treatment of pancreatic islets from mice aged 20-22 weeks induced an increase in the apoptotic nuclei compared to control islets. However, when islets from young mice (5-7weeks) were treated, the number of apoptotic nuclei was clearly reduced. These effects were stronger at lower concentrations of estradiol [72].

In addition to estrogens, other substances can modulate the K_{ATP} channel. Some "phytoestrogens" or endocrine-disrupting chemicals (EDCs) like BPA perturb ion channels in these cells mimicking the actions of the natural hormone, described above [13, 72, 73]. It is well accepted that EDCs exert their actions binding to the classical ERα and ERβ [74, 75] but most of the effects described through the classical pathway

occur at supraphysiological concentrations. In the same way as estrogens, EDCs can modulate β-cell function through alternative pathways [75-77].

Recently, it has been demonstrated that 1 nM BPA has a rapid insulinotropic effect modulating K_{ATP} channel in mice and human β-cells. Application of environmentally relevant doses of BPA rapidly decreased K_{ATP} channel activity, stimulating insulin secretion[13].

Finally, less is known about whether estrogen or BPA can modulate other ion channels present in pancreatic β-cell membranes. It has been demonstrated that physiological concentrations of estradiol directly potentiated L-type Ca^{2+} channels in hippocampal neurons [78]. Rapid effects of environmental pollutants, such as 4-octylphenol, nonylphenol, and *o*, *p'*-DDT, inhibited L-type Ca^{2+} channels in smooth muscle cells [79].This channel is present in pancreatic β-cell but until now it has not been tested for estrogen or xenoestrogen actions on this cell type.

9. Concluding remarks

BPA is an EDC ubiquitously present in human populations of advanced countries, acting as a xenoestrogen and associated in epidemiological studies to the alteration of many physiological functions, being the evidence strong for metabolism, reproduction, development and neuroendocrine regulation. In these physiological processes, ion channels are of crucial importance as they initiate or act as effectors in many signaling pathways. We review here the reported effects of BPA on ion channels in the literature and come to the conclusion that there is evidence that BPA alters ion channel function in excitable cells. This is a crucial mechanism to understand the alterations that BPA exerts in nervous system development as well as in other excitable cells in endocrine and cardiovascular systems.

Nevertheless, the number of publications reporting the effect of BPA on these important signaling molecules in different cell types is still scarce. Approximately, half of these reports used low doses of BPA usually found in humans that could account for some of the molecular mechanism linking BPA exposure to health related problems, as epidemiological studies suggest. The rest of reports, however, employed high doses of BPA not usually found in humans and hence not contributing to clarify these

mechanisms. We conclude that several studies demonstrate that BPA modulates the function of numerous ion channels that play a central role on several neurological, endocrine and metabolic diseases. More systematic mechanistic research on ion channel-mediated BPA effects at environmentally relevant doses is still needed.

Ref	Animal species	Tissue	BPA administration	Ion channel type	Measurement technique	Effect	Cell signalling	Environmental relevant dose	Proposed physiological system affected
[31]	Mice ♂	Testis Epididymis	100 µg/Kg/d (30 d). Intragastric	T-type LVA Ca^{2+} channel (Cav3.1, Cav3.2, Cav3.3)	mRNA expression	↑ (Cav3.1, Cav3.2, Cav3.3) (testis) ↑ (Cav3.1, Cav3.2, (Epididymis) ↓Cav3.3) (Epididymis)	ERβ testis and Cav3.3 epididymis ERα & β (Cav3.1, Cav3.2, epididymis	Yes	Male reproductive function
[26]	Mice ♂ & ♀	Cardiac (left ventricle)	5-5000 µg/Kg/d (90 d). Diet	-Aqp7 (aquaporin 7) -Cacnα2δ2 (auxiliary subunit of HVA Ca^{2+} channel) -ClC1 (Cl^- voltage-gated channel) -Kv1.1 (K^+ voltage-gated channel, shaker-related) -Kv1.6 (K^+ voltage-gated channel, shaker-related) -Mirp3 (β subunit of K^+ voltage-gated channels) -Kir4.1 (K^+ inward rectifier channel) -Navβ4 (β subunit of Na^+ channels) -Nav2.3 (Na^+ voltage-gated channel)	mRNA expression	-↑ ♂ -↓ ♂ -↓ ♂ -↓ ♂ -↓ ♂ -↓ ♂ -↑ ♀ -↑ ♀	N/M	Yes	Cardiovascular system
[56]	Mice ♂ & ♀	Brain (hippocampus)	4-40000 µg/Kg/d (84 d). Oral	$GABA_{A2}$	Protein levels (Western blot)	-↑ ♀ -↓ ♂	N/M	Yes ♀ No ♂	Brain (anxiety & depression)
[28]		Transfected cells (AD 293)	1-100 µM (acute application)	hSlo α and β1 subunits of BK channel (K^+ voltage- & Ca^{2+}-gated channel)	Electrophysiology	-↑	Direct opening	No	Several
[32]	Human	Transfected cells (HEK 293)	0.1nM-100 µM (acute application)	T-type LVA Ca^{2+} channel (Cav3.1, Cav3.2, Cav3.3)	Electrophysiology	-↓	N/M	Yes Cav3.2 No Cav3.1/3.3	Brain Cardiovascular system
[36]	Rat	Cardiac (ventricular myocites)	1 nM (acute application)	RyR (ryanodine receptor)	Protein levels (Western blot)	-↑	ERβ (PKA)	Yes	Cardiovascular system (arrhythmias)
[41]	Mice ♂ (trans-generational protocol)	Testis Epididymis	0.1-10-1000 ppm (drinking water) to generation F0 (30 d) and F1-F2 for the entire experimental period	-Cftr (Cl^- channel; Cystic fibrosis transmembrane conductance regulator) -Aqp1 (aquaporin 1) -Aqp9 (aquaporin 1)	mRNA expression	-↑ (0.1-1000 ppm) -↑ (0.1-1000 ppm) -↑ (10 ppm)	N/M	Yes (0.1 ppm-10 ppm)	Male reproductive function (infertility)

Ref	Animal species	Tissue	BPA administration	Ion channel type	Measurement technique	Effect	Cell signalling	Environmental relevant dose	Proposed physiological system affected
[50]	Mice ♂ & ♀	Brain (hippocampus)	400, 4000 ,40000 µg/Kg/d (84 d). Oral	-GluN1 (NMDA) -GluR1 (AMPA)	Protein levels (Western blot)	-↓ -↓	N/M	Yes	Brain
[51]	Mice ♂ (trans-generational protocol)	Brain (hippocampus)	400-40000 µg/Kg/d (from GD7 to PND21). Oral	-GluN1 (NMDA) -GluR1 (AMPA)	Protein levels (Western blot)	-↓ -↓	N/M	Yes No	Brain
[38]		Transfected cells (LNCaP & PC-3 PCa)	1-10 nM (24-48 h incubation)	Orai1	Protein levels (Immunofluorescence and Western blot) Ca2+ fluorescence	-↑	N/M	Yes	Cancer cells (Prostate)
[33]	Rat GH3, DRG and cardiac myocytes & HEK 293 cells	Native and transfected cells	10-70 µM (acute application)	-L, P/Q and R-type HVA Ca^{2+} channels (GH3) -N and T-type HVA Ca^{2+} channels (DRG) -L-type HVA Ca^{2+} channels (cardiac myocytes) -R-type HVA Ca^{2+} channels (HEK)	Electrophysiology	-↓ -↓ -↓ -↓	Direct block	No	Endocrine, cardiac and neuronal cells
[37]	Pregnant Mice ♀	Kidney Duodenum	5000-50000 µg/Kg/d (from GD6.5 to GD16.5). Oral	-TRPV6 -TRPV5	mRNA expression Protein levels (Western blot)	-↓ -↓	N/M	No	Ca^{2+} homeostasis
[24]		Transfected cells (HEK 293)	1-10000 µM (acute application)	hNav1.5 (Na^+ voltage-gated channel)	Electrophysiology	-↓	Direct block	No	Cardiovascular system
[13]	Mice ♀ & Human islets	Pancreatic β-cells	1 nM (acute application)	Kir6.2 (K_{ATP} channel)	Electrophysiology	-↓	ERβ	Yes	Endocrine System
[49]	Rat ♂	Brain (hippocampus)	50-500 µg/Kg (1-24 h treatment)	GluN1 & GluN2B (NMDA)	Protein levels (Western blot)	-↑ (1h treatment) (No effect 24h treatment)	ERs (non-genomic effects; phosphorylation)	Yes	Brain
[55]	Rats ♀ & ♂	Brain (amigdala)	2 µg/Kg/day (subcutaneous from GD10 to PND7)	$GABA_A$	Electrophysiology	-↓	N/M	Yes	Brain
[25]	Mice ♂	Dorsal Root Ganglion neurons	40µM (acute application)	-TTX-sensitive Na^+ channels -TTX-resistant Na^+ channels	Electrophysiology	-↓ -↓	-Direct block -Block (dependent on PKC & PKA)	No	Neuronal cells

Ref	Animal species	Tissue	BPA administration	Ion channel type	Measurement technique	Effect	Cell signalling	Environmental relevant dose	Proposed physiological system affected
[48]	Rats	Brain (hippocampus)	1-1000 nM (acute application)	GluN2B (NMDA)	Immunocytochemistry Protein Levels (Western blot)	-↑	ERβ (phosphorylation)	Yes	Brain
[47]	Rats ♀ & ♂	Brain (hippocampus)	50-200000 µg/Kg/day (oral exposure from GD7 to PND21)	GluN1, GluN2A & GluN2B (NMDA)	Protein levels (Western blot)	-↓ (low & high doses)	ERβ	Yes	Brain
[27]	Human & canine cells AD-293 cells	coronary smooth cell	10-100µM (acute application)	hSlo α and β1 subunits of BK channel (K^+ voltage- & Ca^{2+}-gated channel)	Electrophysiology	-↑	Direct opening (β_1 subunit)	No	Cardiovascular system
[46]	Mice ♀ & ♂	Brain (hippocampus)	500,5000 & 50000 µg/Kg/day (oral exposure from GD7 to PND21)	GluN1, GluN2A & GluN2B (NMDA)	Polyacrylamide gel electrophoresis	-↓	N/M	No	Brain
[45]	Mice ♀ & ♂	Brain (hippocampus)	100 & 500 µg/Kg/day (oral exposure from GD7 to PND21)	NMDA Receptor (not subtype specified)	Autorradiographic receptor binding assay	-↓	N/M	Yes	Brain
[35]	Mice ♂	Sertoli cells	5-60µM (acute application)	RyR	Ca^{2+} fluorescence	↑ RyR (0.4-4µM)	Direct opening	No	Reproductive system
[54]	Rat	Brain (hippocampus)	3-300 µM (acute application to dissociated)	$GABA_A$	Electrophysiology	-↑ (low [GABA]) -↓ (high [GABA])	Direct	No	Brain
[34]		PC12 cells (adrenal gland tumour)	25-150 µM (acute application)	-N-type HVA Ca^{2+} channels -RyR	Measurement of dopamine release with HPLC	-↑ -↑	Non genomic action on G-proteins and PKA activation	No	Cancer cells (Adrenal)
[44]	Rat	Organotypic hippocampal slice cultures (PD8)	1 nM (24 h incubation)	GluN1 (NMDA)	Immunohistochemistry	-↑ (expression) in CA3	No mediated by ERs	Yes	Brain
[30]	Human	Airway epithelial Calu-3 cells	100 µM (30 min pretreatment of cells)	-K_{cAMP} (K^+ voltage-gated channel, slow rectifier modulated by cAMP) -K_{Ca} intermediate conductance	Electrophysiology	-↓ -↓ (no effects in 12-72 h of BPA incubation)	Direct block	No	Respiratory system

Ref	Animal species	Tissue	BPA administration	Ion channel type	Measurement technique	Effect	Cell signalling	Environmental relevant dose	Proposed physiological system affected
[52]	Human	Transfected cells (*Xenopus* oocytes)	0.1-100 µM (acute application)	Nicotinic receptors α3β4 & α4β2	Electrophysiology	-↓	Direct block	No	Brain
[53]	Rat & Bovine	Transfected cells (*Xenopus* oocytes)	0.1-1 mM (acute application)	$GABA_A$	Electro physiology	-↑ (0.1 mM BPA) -↓ (1 mM BPA)	Direct	No	Brain

Table 1. **Summary of the effects of BPA on ion channels reported in the literature**.

The numbers in the Reference column (Ref) refer to the study cited in the Bibliography. The effect of BPA on ion channel is indicated with an up arrow (↑) or a down arrow (↓) for an upregulation of the activity or expression of the ion channel or a downregulation of the activity or expression of the ion channel, respectively. Abbreviations: BPA: Bisphenol A; ER: Estrogen receptor; GD: gestation day; HVA: High voltage-activated; LVA: Low voltage-activated; N/M: Not measured; PND: post-natal day; NMDA: N-methyl-D-aspartate; RyR: Ryanodine receptor.

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Figure 1.Hypothetical cell expressing all ion channels modulated by BPA. Up arrows indicate upregulation of the activity or expression of the ion channel by BPA and down arrows indicate a downregulation of the activity or expression of the ion channel by BPA. Numbers indicate the reference where these effects were reported (see bibliography).