ORIGINAL ARTICLE

Chronic Exposure to High Doses of Bisphenol A Exhibits Significant Atrial Proarrhythmic Effects in Healthy Adult Rats

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ABSTRACT

Objectives: We aimed to evaluate the effects of chronic exposure to bisphenol A (BPA) on atrial fibrillation (AF) occurrence in rats.

Methods: Twenty-two healthy female Wistar rats were randomized into three groups: Control (no BPA; n=7), BPA (exposed to usual BPA doses; 50 µg/kg/day, 9 weeks; n=7), and hBPA (exposed to high BPA doses; 25 mg/kg/day, 9 weeks; n=8). 24-h ECG monitoring was performed using radiotelemetry ECG devices prior to and after transesophageal atrial pacing. Spontaneous and pacing-induced atrial arrhythmias, autonomic tone, and *in vivo* an *in vitro* atrial arrhythmogenicity-related parameters were evaluated.

Results: All studied parameters were similar between Control and BPA (all p>0.05). However, compared to Control, hBPA presented more atrial premature beats both at baseline (p=0.04) and after pacing (p=0.03), more AF episodes (p<0.001) and of longer duration (p=0.02) following transesophageal stimulation, and significantly higher vagal tone (all p<0.05).

Conclusions: Chronic exposure to high, but not usual BPA doses induced significant atrial proarrhythmic effects in healthy rats, and this may be at least partially due to BPA-induced vagal hyperactivation. Exposure to high BPA doses, such as that occurring in plastics industry workers, could favor AF occurrence even in the absence of underlying cardiovascular disease.

Keywords: atrial fibrillation, bisphenol A, heart rate variability, vagal hyperactivation.

REZUMAT

Obiective: Ne-am propus să evaluăm efectele expunerii cronice la bisfenol A (BPA) asupra apariției fibrilației atriale (FA) la șobolan.

Metode: Șobolani femele Wistar au fost randomizați în trei grupuri: Control (fără BPA; n=7), BPA (expunere la doze uzuale de BPA; 50 µg/kg/zi, 9 săptămâni; n=7) și hBPA (expunere la doze mari de BPA; 25 mg/kg/zi, 9 săptămâni; n=8). Pentru fiecare animal s-a efectuat monitorizare ECG/24 de ore înainte și după stimulare electrică transesofagiană. Au fost evaluate aritmiile atriale spontane și induse prin stimulare, tonusul autonom și parametrii de aritmogenicitate atrială, *in vivo* și *in vitro*.

Rezultate: Toți parametrii studiați au fost similari între Control și BPA (toate p>0,05). Comparativ cu grupul Control, hBPA a prezentat, însă, mai multe extrasistole atriale atât bazal (p=0,04) cât și după stimulare (p=0,03), episoade de FA mai numeroase (p<0,001) și de durată mai lungă (p=0,02) post-stimulare și tonus vagal mai ridicat (toate p<0,05).

Concluzii: Expunerea la doze mari de BPA a indus efecte proaritmice atriale la șobolani, probabil cel puțin parțial datorită hiperactivării vagale induse de BPA. Expunerea la doze mari de BPA, ca cea întâlnită la lucrătorii din industria materialelor plastice, ar putea favoriza apariția FA chiar și în absența unei boli cardiovasculare subiacente.

Cuvinte cheie: bisfenol A; fibrilație atrială; hiperactivare vagală; variabilitatea ritmului sinusal.

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INTRODUCTION

The risk of developing cardiovascular diseases, including cardiac arrhythmias, increases considerably with advancing age[1]. Atrial fibrillation (AF) is the most common sustained heart rhythm disorder, affecting between 2% and 4% of the world population according to recent data[2]. The continuous increase in AF prevalence has led to predictions about the magnitude of the AF burden in the future. Krijthe et al. anticipated that in the European population the prevalence of AF will increase to 2.5% by 2030 and to 3.5% by 2060[3]. Even though the world population is progressively older[4] and the prevalence of numerous factors known to be involved in AF onset and maintenance is on the rise[5,6], these factors can only partially explain the continuous increase in AF prevalence recorded over the past decades, suggesting that other, yet unidentified factors may also contribute to this worrisome epidemiologic progress[7].

Bisphenol A (BPA), one of the most extensively used chemical compounds, is widely utilized in the production of epoxy resins and polycarbonate plastics. Since these materials are present in a vast variety of common products, including in plastic bottles, food containers and cans, dental materials, water pipes, toys, protective equipment, electronics, and even in numerous medical devices, human exposure to BPA via different routes (i.e., oral, cutaneous, or by inhalation) is both important and continuous[8]. In addition, the presence of BPA monomers that remain unbound during the production process makes these BPA molecules easily transferable to the human body even during common exposure[9]. Studies indicated the presence of BPA in the urine of more than 90% of the general population, with higher BPA concentrations in workers in the plastics industry[10].

The main health implications of BPA derive from its role as an estrogen endocrine disruptor. Accumulating data from both human[11] and animal[12] studies incriminate BPA exposure as an important contributor to a vast variety of diseases, including diabetes mellitus, obesity, and various malignancies. Studies have also suggested a potential link between BPA exposure and cardiovascular diseases such as arterial hypertension, ischemic heart disease, and ventricular arrhytmias[11]. However, no study has assessed to date the potential impact of BPA exposure on atrial arrhythmogenicity. Moreover, even the BPA-related cardiotoxicity remains a matter of debate. Although several cross-sectional clinical studies support a relationship between BPA exposure and cardiovascular disease[10,13], such studies cannot ascribe causation, whereas preclinical studies have mostly focused on *in vitro* testing, using acute exposure to doses exceeding those observed following common human exposure[14,15].

Accordingly, we aimed to evaluate the effects of chronic exposure to both high BPA concentrations and BPA concentrations relevant for common human exposure on atrial electrophysiological parameters, on cardiac autonomic modulation, and on atrial spontaneous and electrically-triggered arrhythmogenicity in healthy adult rats.

MATERIALS AND METHODS

Studied animals

22 adult female Wistar rats (263 ± 12.54 g) were randomly divided into three groups: Control (n = 7), BPA (n = 7), and hBPA (n = 8). Animals in the BPA group were exposed to BPA doses of 50 μg/kg of body weight/day, considered, according to previous studies[15], relevant for common human exposure[16]. In accordance with previous studies[17], animals in the high BPA (hBPA) group were administered 25 mg/kg of body weight of BPA/day. Bisphenol A (Sigma-Aldrich, St. Louis, MO) was administered in all rats in the drinking water for 9 consecutive weeks. Control rats received normal tap water throughout the study. All protocols used in the present study complied with the International Council for Laboratory Animal Science guidelines (Directive 2010/63/EU) and were approved by the local Ethics Committee.

Body weight and systolic blood pressure assessment

Body weight was determined weekly for all rats throughout the study. Systolic blood pressure (SBP) was assessed in all rats by photoplethysmography. Briefly, a pneumatic cuff was placed proximally on the rats' tail and was inflated/deflated using a programmable electro-sphygmomanometer (PE-300; Narco Bio-Systems, Austin, TX). The photoplethysmography sensor was placed distally, at the level of the caudal artery. The cuff and photo-transducer signals were routed to a computer equipped with an acquisition board. Signals were recorded and analyzed using a program developed using the LabVIEW 8.6 software (National Instruments, Austin, TX). Each animal underwent SBP measurement after eight weeks of BPA or normal water exposure.

Electrocardiographic monitoring and heart rate variability analysis

After six weeks of BPA or normal water administration, all animals were implanted under isoflurane inhalation anesthesia (4 L/min, 2.5%) with an electrocardiographic radiotelemetry transmitter (Data Science International, St. Paul, MN). The transmitter was placed in a dorsal, subcutaneous pocket, and the two ECG leads were tunneled subcutaneously and secured in a lead II configuration. Continuous 24-h ECG monitoring was performed in freely moving rats and all ECG traces were analyzed using a dedicated software.

The mean heart rate and the occurrence and duration of spontaneous atrial arrhythmic events (i.e., number of atrial premature beats/24-h, and number and duration of AF episodes/24-h) were assessed at baseline and after application of an atrial electrical stimulation protocol, as described below. Atrial premature beats were defined as early P waves, with morphology different than the sinus rhythm P waves, followed by narrow QRS complexes. Spontaneous AF was defined as the occurrence of three or more consecutive irregular, supraventricular beats (i.e., irregular ventricular response with narrow QRS complexes), in the absence of clearly identifiable P waves.

Continuous ECG recordings obtained prior to atrial electrical stimulation were also used for heart rate variability (HRV) analysis in both the time and frequency domains. The HRV parameters were determined by analyzing beat-to-beat variations in RR intervals, as described previously.[18] The standard deviation of normal RR intervals (SDNN), the root mean square of the successive RR interval differences (RMSSD), and the percentage of successive RR intervals that differed by more than 5 ms (pNN5) were assessed as time domain parameters, whereas the low-frequency (LF; 0.3–0.6 Hz) and the high-frequency (HF; 0.6–2.5 Hz) components of the HRV spectrum and the LF/HF ratio were assessed as frequency domain parameters.

Electrical *in vivo* transesophageal atrial burst pacing

Atrial fibrillation inducibility was assessed *in vivo* in anesthetized rats (ketamine/medetomidine – 75.0/0.5 mg/kg, i.p.), as described previously[19]. All rats underwent a single transesophageal stimulation protocol using a 5 F bipolar lead, at a frequency of 4,000 stimuli/min (stimulus duration 6 ms), at the minimum voltage required to ensure atrial capture. Stimulation was applied for 15 consecutive cycles of 20 s each, with 5-min free intervals between the successive stimulations. Surface ECG was recorded throughout the stimulation protocol, as described previously,[19] and AF inducibility (i.e., the presence and the number of AF episodes induced by transesophageal atrial burst pacing) was assessed.

In vitro assessment of atrial action potentials

At 24 h after the stimulation protocol, the animals were euthanized, the hearts were rapidly excised, and placed in oxygenated Krebs-Henseleit solution (NaCl 118.00 mM, KCl 4.70 mM, $NaHCO_3$ 25.00 mM, $MgSO_4$ 1.20 mM, $CaCl_2$ 1.25 mM, KH_2PO_4 1.20 mM, and glucose 11.00 mM). The left atrium was isolated and transferred into the Steiert organ bath, where it was continuously perfused with oxygenated Krebs-Henseleit solution at 37°C. The atrial tissue was electrically stimulated at a frequency of 1 Hz and the transmembrane atrial action potentials were recorded using a microelectrode filled with 3 M KCl (resistance 2-4 MΩ). Atrial action potentials were recorded at baseline and after exposure to proarrhythmogenic stimuli (i.e., 10^{-5} M adrenaline solution, 10^{-5} M acetylcholine solution, and 1.25 mM $CaCl_2$ solution), with 15-min washing intervals between the different solutions. For each stage of the protocol, 10 action potentials were analyzed, and the depolarization rate and the action potential duration until reaching 50% (APD50) and 90% (APD90) of complete repolarization were assessed, as described previously[20].

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 8.0.2 (GraphPad Software; San Diego, CA). Given the low sample size, all between-group comparisons were performed using the non-parametric Mann-Whitney test. Multiple comparisons were performed using Kruskal-Wallis test. The results are reported as median and interquartile range. The p value was set at 0.05 for statistical significance.

RESULTS

Effects of chronic bisphenol A exposure on body weight, systolic blood pressure, and heart rate

At the end of the study, both groups exposed to BPA showed lower body weight than the Control rats (both $p = 0.04$), and this effect was not affected by the BPA dose (Figure 1). No significant difference was found in either the heart rate or the SBP values between the Control and the BPA, nor between the Control and the hBPA groups (all $p > 0.05$; Table 1).

Table 1. Systolic blood pressure and mean 24-h heart rate in Control rats and in rats exposed to usual (BPA) and high (hBPA) bisphenol A doses for 9 weeks

Parameter	Control (n = 7)	BPA (n = 7)	hBPA (n = 8)	p-value
SBP (mmHg)	105.0 (87.5-117.5)	110.0 (100.0-127.5)	110.0 (110.0-120.0)	0.46
HR (bpm)	362.6 (348.4-406.5)	364.1 (354.9-375.6)	352.4 (347.1-364.6)	0.55

The values are expressed as median and interquartile range; p-values refer to between-group comparisons based on the Kruskal-Wallis test.
HR – heart rate; SBP – systolic blood pressure

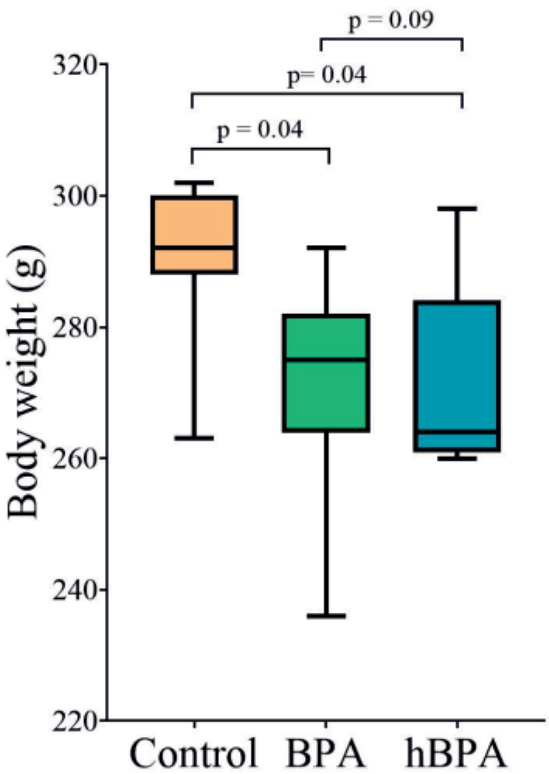

Figure 1. Body weight in Control rats and in rats exposed to usual (BPA) and high (hBPA) bisphenol A doses for 9 weeks. Data are expressed as median and interquartile range; p-values were calculated using the Mann-Whitney test.

Chronic high-dose bisphenol A exposure increases cardiac parasympathetic activity

Heart rate variability analysis revealed significantly higher HF (p = 0.001) and LF (p = 0.04) components of the HRV spectrum in the BPA compared to the Control rats, while no significant difference was observed in the LF/HF ratio (p = 0.71), nor in the time domain HRV parameters (all p >0.05) between the two groups (Table 2). Meanwhile, rats in the hBPA group presented significantly higher RMSSD, pNN5, and HF components of the HRV spectrum, reflecting higher parasympathetic activity, compared to the Control rats (all p< 0.05; Table 2). In addition, the LF/HF ratio was significantly lower in the hBPA compared to the Control rats (p = 0.02), reflecting a shift of the autonomic activity toward vagal dominance in the hBPA rats (Table 2).

Chronic bisphenol A exposure does not affect atrial action potential parameters

Regardless of the dose, BPA exposure had no significant effect on baseline action potential parameters (Table 3); i.e., the depolarization rate, APD50, and APD90 were all similar between the BPA and the hBPA groups, and the Control group, respectively (all p >0.05). Action potential changes in response to adrenaline, acetylcholine, and Ca^{2+} overload were also similar between the BPA and the hBPA groups, and the Control group (all p >0.05).

Chronic high-dose bisphenol A exposure increases spontaneous atrial arrhythmogenicity

The occurrence and the number of electrically-induced AF episodes was not significantly different between the BPA and the hBPA groups, and the Control group, respectively (all p >0.05). Also, there was no significant difference in the number of atrial ectopic beats or in the number and duration of spontaneous AF episodes either at baseline or during the post-electrical stimulation period between the Control and the BPA groups (all p >0.05; Figure 2). However, hBPA rats presented a significantly higher number of atrial ectopic beats (p = 0.04), but not of spontaneous AF episodes (p = 0.60) at baseline compared with the Control rats (Figure 2). Moreover, during the period following the *in vivo* transesophageal atrial pacing, hBPA rats presented a significantly higher number of both atrial ectopic beats (p = 0.03) and of AF episodes (p< 0.01), as well as significantly longer duration of spontaneous AF episodes (p = 0.02) compared with the Control rats (Figure 2).

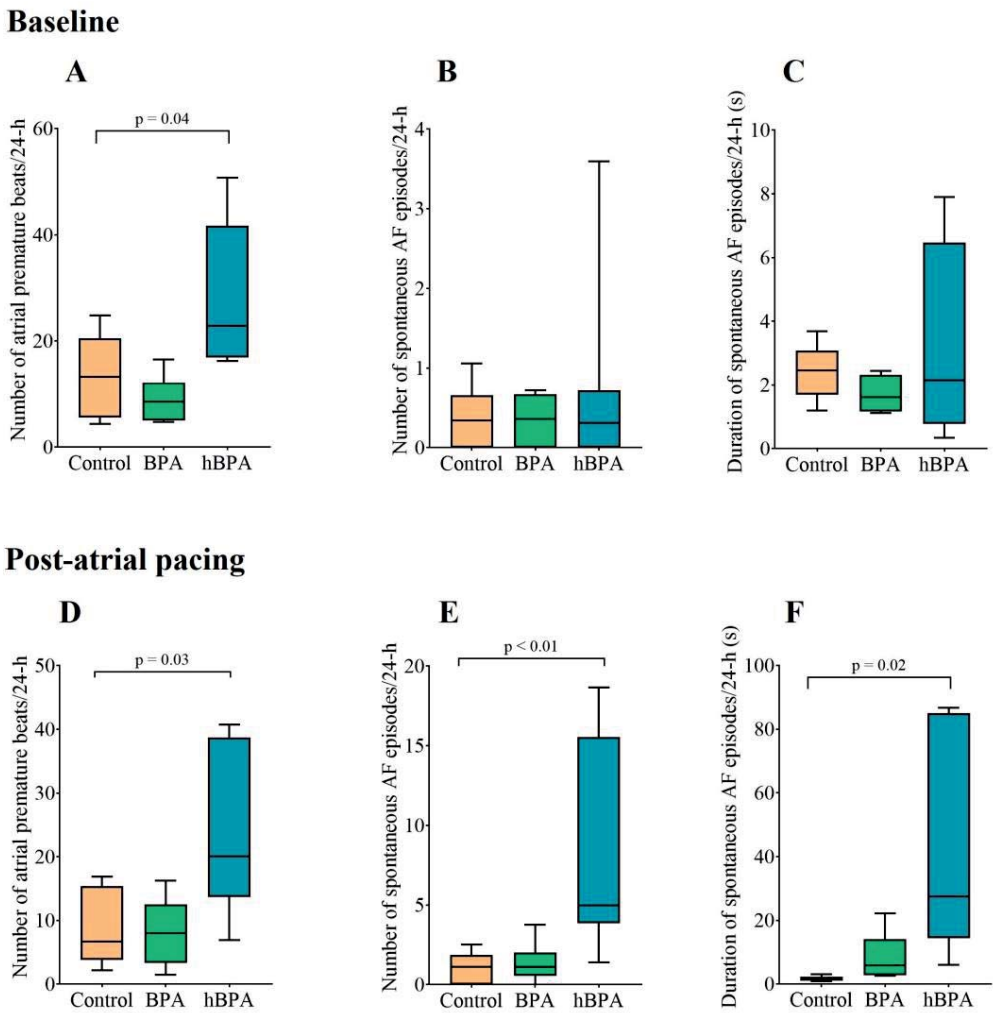

Figure 2. Spontaneous atrial arrhythmogenicity at baseline and after transesophageal atrial burst pacing in Control and in rats exposed to usual (BPA) and high (hBPA) bisphenol A doses for 9 weeks. The figure illustrates the number of atrial premature beats (A and D), the number (B and E) and the duration (C and F) of spontaneous atrial fibrillation (AF) episodes per 24-h at baseline (upper panels – Baseline) and during the period following the *in vivo* transesophageal atrial pacing protocol (lower panels – Post-atrial pacing). The values are expressed as median and interquartile range; p-values refer to between-group comparisons based on the Mann-Whitney test.

Table 2. Heart rate variability parameters in Control rats and in rats exposed to usual (BPA) and high (hBPA) bisphenol A doses for 9 weeks

Parameter	Control (n = 7)	BPA (n = 7)	hBPA (n = 8)	p-value Control vs. BPA	p-value Control vs. hBPA
Time domain					
SDNN (ms)	21.12 (15.71-22.03)	20.66 (18.93-26.77)	23.54 (21.16-28.11)	0.46	0.10
RMSSD (ms)	3.43 (3.14-4.16)	4.28 (4.13-4.71)	4.37 (4.93-5.54)	0.16	0.03
pNN5 (%)	11 (11-20)	19 (19-22)	22 (17-31)	0.14	0.04
Frequency domain					
LF (ms^2)	1.70 (1.68-1.91)	2.76 (2.06-3.12)	3.01 (1.21-4.73)	0.04	0.63
HF (ms^2)	5.37 (4.69-5.94)	9.06 (7.89-9.97)	9.22 (7.60-11.68)	0.001	0.001
LF/HF	0.36 (0.28-0.38)	0.35 (0.25-0.37)	0.24 (0.17-0.29)	0.71	0.02

The values are expressed as median and interquartile range; p-values refer to between-group comparisons based on the Mann-Whitney test.
HF – high-frequency (0.6-2.5 Hz) signals; LF – low-frequency (0.3-0.6 Hz) signals; LF/HF – the ratio of low- to high-frequency components; pNN5 – percentage of successive RR intervals that differed by >5 ms; RMSSD – root mean square of successive RR-interval differences; SDNN – standard deviation of normal RR intervals

Table 3. Atrial action potential parameters at baseline in Control rats and in rats exposed to usual (BPA) and high (hBPA) bisphenol A doses for 9 weeks

Parameter	Control (n = 7)	BPA (n = 7)	hBPA (n = 8)	p-value
Depolarization rate (mV/s)	26.2 (19.1-41.6)	26.0 (18.7-40.0)	21.8 (15.3-26.3)	0.39
APD50 (ms)	14.0 (12.9-19.9)	14.4 (13.9-16.7)	17.4 (15.6-23.3)	0.10
APD90 (ms)	37.4 (35.2-43.6)	37.9 (32.5-43.2)	43.4 (42.6-52.5)	0.16

The values are expressed as median and interquartile range; p-values refer to between-group comparisons based on the Kruskal-Wallis test.
APD50 – action potential duration until reaching 50% of complete repolarization; APD90 – action potential duration until reaching 90% of complete repolarization

DISSCUSIONS

Chronic bisphenol A exposure leads to lower body weight, but has not effect on heart rate or arterial blood pressure

In the present study, BPA exposure was associated with significantly lower body weight, independently of the dose. To date, several clinical epidemiological studies indicated an association between BPA exposure and weight gain[21-23]. However, although all those studies showed a link between urinary BPA levels and obesity, neither of them was able to establish a cause-effect relationship. Contrarily, in a prospective study conducted on elderly individuals, the authors found no significant association between BPA serum concentrations and fat mass or distribution assessed by imaging techniques[24]. Meanwhile, early prepubertal exposure of female mice to high doses of BPA was associated with lower body weights compared with control, but no effect of BPA on body weight was observed during adulthood[25]. The lower body weight gain related to chronic exposure to BPA observed in the present study in healthy adult female rats is most likely the consequence of β-estrogen receptors activation leading to alterations in adipose tissue metabolism and increase in energy consumption[26].

The data in the literature regarding the effect of BPA on blood pressure are also highly controversial. While several epidemiological studies showed an association between BPA exposure and high blood pressure[27-29], others showed, on the contrary, a negative correlation between the two[30]. Belcher et al.[31] showed a significant decrease in blood pressure following BPA exposure in CD-1 mice, independently of gender, whereas in their *in vivo* study in young mice, Saura et al.[32] showed an endothelial dysfunction-mediated increase in blood pressure even at low-dose BPA exposure. In the present study, exposure to BPA of adult healthy female rats did not show any significant effect on SBP, suggesting that, if present, the effect of BPA on SBP is likely to be linked to other factors, such as gender, age, or BPA exposure duration. Data regarding the impact of BPA on heart rate are very scarce. In a clinical study conducted on 521 elderly citizens, Bae et al.[28] showed an increase in mean heart rate with increasing urinary BPA concentrations. In our study, however, mean heart rate was not significantly influenced by BPA exposure in adult female rats.

Bisphenol A exposure and atrial arrhythmias

Even though several clinical studies investigated the link between BPA exposure and cardiovascular diseases, little is known to date regarding the mechanisms that could underlie the potential cardiovascular toxicity of this extensively used chemical compound. It has been suggested that the potential toxic effects of BPA could be related to its function as an endocrine disruptor[33]. The protective role of endogenous estrogens on the cardiovascular system is well known, but the effects of exogenous estrogen administration remain highly controversial[34].

Data regarding the cardiovascular toxicity of BPA are extremely limited. Several epidemiological studies demonstrated significant associations between BPA exposure and various cardiovascular diseases. None of those studies could establish, however, a causal BPA-cardiovascular disease relationship. Meanwhile, experimental studies, most of them conducted *in vitro,* have only evaluated the effects of acute BPA exposure on the cardiovascular system, often using much higher doses than those identified in usual human exposure. Further investigation is therefore needed in order to establish the exact cardiotoxic potential of BPA and to identify the mechanisms that could underlie such an effect.

The increasing prevalence of AF[2] in the past decades raises serious questions about a potential atrial proarrhythmogenic effect of environmental factors, including BPA. The mechanisms that underlie AF onset and persistence are complex and remain incompletely elucidated. Broadly, these mechanisms rely on ectopic activity and re-entry[35], occurring as a result of

electrical remodeling, modulated by changes in various ion currents, structural remodeling, via fibrosis, and autonomic remodeling, expressed as an alteration in sympathetic and/or vagal tone[36-38].

In previous experimental studies, acute exposure to high doses of BPA was associated with ventricular proarrhythmic effects. In female rodents, BPA exposure increased ventricular arrhythmogenicity in both *in vitro* and *in vivo* studies[14,15]. Estrogen receptor signaling, calcium handling alterations, and direct effects of BPA on cardiac ion channels have been incriminated as main molecular mechanisms involved in these BPA-related proarrhythmic effects. Acute, high-dose BPA exposure has been shown to promote phospholamban phosphorylation and to increase Ca^{2+} reuptake by the sarcoplasmic reticulum via the sarco/endoplasmic reticulum Ca^{2+}-ATPase 2A, thus favoring the occurrence of calcium sparks[14,39]. Data in the literature have also suggested that high-dose BPA exposure interferes with multiple ion channels functioning. *In vitro,* high-dose BPA was shown to block Nav1.5 channels by binding to local anesthetics receptor site[40], and to exhibit inhibitory effects on L-type Ca^{2+} channels by acting on a biding site located in the extracellular region of the channels[41]. As these mechanisms underlie not only ventricular arrhythmias, but also AF, one would expect BPA to also exert atrial proarrhythmic effects.

Additionally, exposure to BPA, regardless of the dose, has been shown to cause morphological changes of the heart. Several studies reported increased myocardial surface area after exposure to various BPA doses, related to either impaired mitochondrial function or up-regulation of genes involved in cardiac hypertrophy[42-44]. High-dose BPA was also shown to trigger myocardial estrogen receptor activation-mediated fibroblast proliferation, collagen production, and consequent fibrosis, and to alter cardiac mechanical function[44,45], which could all contribute to increased cardiac arrhythmogenicity.

To the best of our knowledge, this is the first study designed to evaluate the effect of BPA exposure on atrial arrhythmogenicity. In the present study, exposure to BPA doses relevant for human exposure did not affect atrial arrhythmogenicity. Meanwhile, in baseline conditions, high BPA doses significantly increased the number of atrial premature beats, but not of spontaneous AF episodes. Although BPA exposure did not seem to affect AF inducibility during transesophageal atrial pacing, high BPA doses induced a significant atrial proarrhythmic effect following electrical stimulation of the atria. A 25 mg/kg/day BPA dose led to a significant increase in the number of atrial premature beats/24-h, as well as in the number and duration of post-electrical stimulation spontaneous AF episodes. These findings suggest that BPA exerts a dose-dependent effect on atrial arrhythmogenicity, with high BPA doses exhibiting not only ventricular[14,15], but also atrial proarrhythmic effects. Our findings therefore suggest that massive exposure to BPA, such as that occurring in workers in the plastics industry, may lead to important atrial proarrhythmic effects. In healthy adult female rats, exposure to human-relevant doses did not appear to produce similar effects. However, it remains to be established whether the same is true in the presence of underlying cardiovascular disease.

The exact mechanisms underlying high-dose BPA-related atrial arrhythmogenicity remain to be established. According to our *in vitro* data, BPA exposure does not seem to exert important effects on the electrical activity of the atria, regardless of the dose. Meanwhile, HRV analysis indicated a significant increase in cardiac vagal modulation (as reflected by the higher parasympathetic indexes RMSSD and pNN5 and the increased HF components of the HRV spectrum) and a significant shift in the sympatho-vagal balance toward vagal dominance (as reflected by the significantly lower LF/HF ratio) in the rats exposed to high BPA doses. To the best of our knowledge, this is the first experimental study designed to investigate the direct effect of BPA exposure on HRV parameters, whereas human data are extremely limited in this regard. Contrarily to our findings, Bae et al.[28] reported a significant decrease in RMSSD with increasing urinary BPA concentrations, indicating an elevated sympathetic activity, in human subjects. Future studies will thus have to elucidate the exact effects that BPA exerts on cardiac autonomic modulation and to assess whether these effects play a role in BPA-related atrial arrhythmogenicity.

Strengths and limitations

To the best of our knowledge, this is the first study designed to investigate, both *in vivo* and *in vitro,* the direct effect of chronic BPA exposure on atrial arrhythmogenicity. Chronic administration of both high-dose and usual-dose BPA was used, making this study much more clinically-relevant than the vast majority of previous studies in this field. The multidisciplinary approach used in this study provided a comprehensive view on the impact of BPA exposure on atrial arrhythmogenicity and atrial arrhythmogenicity-related

parameters. The present study indicates BPA-induced vagal hyperactivation as potential mechanism for the significant increase in spontaneous atrial arrhythmogenicity observed in rats exposed to high BPA doses. However, further studies are needed to explore other potential mechanisms responsible for this effect. Due to the small size of the rat atria, additional tissue analyses could not be performed; evaluation of potential BPA-induced structural atrial alterations would have been of interest. Finally, no significant change in atrial arrhythmogenicity was observed in the rats exposed to usual BPA doses. However, this does not exclude a potential BPA-induced increase in atrial arrhythmogenicity in the setting of structurally remodeled atria. Further studies will have to assess the effects of chronic BPA exposure in the presence of underlying cardiovascular disease.

CONCLUSIONS

The present study demonstrates that chronic exposure to high doses of BPA exhibits significant atrial proarrhythmic effects in healthy adult female rats, suggesting that important accidental or occupational BPA exposure could lead to serious cardiovascular effects, including in healthy individuals. Our data indicate BPA-induced vagal hyperactivation as a potential mechanism underlying this BPA-related atrial proarrhythmic effect. Exposure to BPA doses relevant for usual human exposure did not have a similar effect. However, future studies remain to establish whether this is also true in the presence of underlying cardiovascular disease.

Compliance with ethics requirements:

The authors declare no conflict of interest regarding this article. The authors declare that all the procedures and experiments of this study respect the International Council for Laboratory Animal Science guidelines (Directive 2010/63/EU), as well as the national law. All protocols were approved by the local Ethics Committee.

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