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ORIGINAL PAPER

Effects of bisphenol AF and bisphenol S on the contractility of the porcine uterus

Aleksandra Łukawska[1], Włodzimierz Markiewicz[1], Tomasz Grabowski[2], Jerzy Jan Jaroszewski[1]

**[1] Department of Pharmacology and Toxicology
University of Warmia and Mazury in Olsztyn, Poland
[2] Department of Inorganic Chemistry
Medical University of Gdansk, Poland**

Abstract

Bisphenols (BPs) are widely used in the manufacturing of polycarbonate plastics and epoxy resins. Many recent studies indicate that bisphenols (BPs) can cause adverse side effects in human, including alterations to the reproductive system, such as hindering conception, polycystic ovarian syndrome, premature puberty or reduced sperm quality. They may raise the risk of breast and ovarian cancers and endometriosis. Since there have been no data in the available literature on the effect of BP analogues, BPAF and BPS on the contractile activity of the uterus, the aim of this research was to determine the effect of these two bisphenols on the porcine myometrium (n=6) collected from immature, cyclic and early pregnant animals. Strips of the myometrium were stimulated with increasing concentrations (10^{-13} to 10^{-1}) of BPAF or BPS, and smooth muscle contractility (tension, amplitude and frequency of contractions) was determined with equipment for measuring isometric contractions. BPAF caused a significant decrease (P<0.05-P<0.001) in all examined parameters in all groups of animals, but the highest changes were observed in the myometrium of the immature pigs. BPS caused the highest decrease in the tension and amplitude (P<0.05-P<0.001) of contractions in the myometrium of early pregnant animals and the frequency of contractions (P<0.05-P<0.001) in the myometrium of immature animals. In conclusion, the results indicate that both BPAF and BPS relax the porcine myometrium, but these changes depend on the physiological status of the animals.

Keywords: bisphenol AF, bisphenol S, myometrium, contractile activity, pig

Włodzimierz Markiewicz, PhD DSc, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland, e-mail: mark@uwm.edu.pl, phone: +48 89 523 37 58

INTRODUCTION

Bisphenol AF (BPAF) and bisphenol S (BPS) are the analogs of bisphenol A (BPA). BPAF-containing polymers such as polycarbonate copolymers, polyimides, polyamides, and polyesters are used in high-temperature composites, electronic materials, and gas-permeable membranes. BPAF is also used in many other specialized polymer applications, including plastic optical fibers and waveguides (Matsushima et al. 2010). In turn, BPS is one of the key ingredients used in the production of polycarbonate plastics and epoxy resins, and many other products, including polyvinyl chloride (PVC), thermal printing paper used for fiscal receipts, plastic food and beverage containers, electronic components and paper coatings (Naderi et al. 2014).

Previous studies have shown that BPA can active estrogen receptors (Matthews et al. 2001) and, consequently, has negative effects on the reproductive tract, predisposing to precocious puberty in girls, impeding the pregnancy in women, and reducing the semen quality in men (Ruan et al. 2015). It has been suggested that BPS has a similar affinity for estrogen receptors (ER) as BPA (Rochester, Bolden 2015), while BPAF shows stronger binding to ER receptors than BPA (Kitamura et al. 2005). This suggests that both BPS and BPAF may affect/impair reproductive function, although knowledge in this area is relatively poor. One study on female zebrafish showed a reduction in egg hatchability and an increase in malformations compared to those not exposed to BPS during the breeding season (Ji et al. 2013). Moreover, Naderi et al. (2014), similarly using the female zebrafish model, observed a decrease in thyroxine and triiodothyronine levels and a decrease in the amount of egg production. An increase in embryo mortality was also observed in experiments using a nematode Caenorhabditis elegans model (Chen et al. 2016). In the case of mammals, an increase in absolute and relative uterine weights under the influence of BPS was observed in immature female rats (Yamasaki et al. 2004). Moreover, exposure to BPS from postnatal day 1 to 10 in female rats has been shown to increase body weight, delay puberty onset and alter estrous cyclicity, decrease uterine weight, decrease the gonadosomatic index, and increase the number of cystic follicles in the ovaries with an increase in the number of atretic follicles, accompanied by an increase in serum testosterone and estradiol levels, but a decrease in the level of progesterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Ahsan et al. 2018).

The effects of BPAF on the reproductive system are poorly understood. To date, BPAF has been shown to increase the incidence of malformations and decrease the survival rate of zebrafish embryos whose mothers were exposed to BPAF (Shi et al. 2015). In addition, BPAF has been observed to increase the uterine weight of Crj:CD (SD) rats (Yamasaki et al. 2003) and Sprague Dawley rats (Conley et al. 2016) and to inhibit in vitro oocyte maturation in mice (Nakano et al. 2016).

Since the available literature lacks any data on the effect of BPS and BPAF on the contractile activity of the uterus, the aim of this study was to determine the influence of these two bisphenols on the tension, amplitude and frequency of contractions of the porcine myometrium collected from immature, cyclic and early-pregnant animals.

MATERIALS AND METHODS

Reagents

Reagents needed for the preparation of the Krebs-Ringer buffer (NaCl, KCl, $CaCl_2$, $MgCl_2$, $NaHCO_3$, NaH_2PO_4 and glucose) were purchased from Chempur (Piekary Śląskie, Poland). Acetylcholine chloride (ACh), BPS, BPAF and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). A 10^{-4} M ACh stock solution was prepared in distilled water, and final dilutions were made in deionized water. A 10^{-1} M BPS and BPAF stock solution was prepared in DMSO, and serial dilutions were made with deionized water on the day of the experiment.

Animals

The experimental groups which were consisted of Large White × Polish Landrace gilts divided into three groups (n = 6 in each). The first group (immature group) consisted of sexually immature gilts aged ok. 6-7 months and weighing 98-106 kg. The second group (cyclic group) consisted of cyclic gilts aged 7-8 months and weighing 116-128 kg on days 12-14 of the estrous cycle (luteal phase). The phase of the estrous cycle was confirmed by ovarian morphology (Akins, Morrissette 1968). The third group (early pregnant) consisted of gilts on days 12-16 of gestation (implantation window) aged 8-9 months and weighing 125-143 kg; the procedure of selection and insemination of gilts from this group has been described earlier (Kamiński et al. 2018) and pregnancy was confirmed by the presence and morphology of embryos in both uterine horns (Anderson 1978). The uteri in all groups were collected immediately after slaughter at a meat processing plant ("TOMUS" Tomasz Reihs, Królikowo, Poland) and transported on ice to the laboratory within 0.5 h. In accordance with Polish (Anonymous, 2015) and European (Anonymous 2010) regulations on the protection of animals used for scientific or educational purposes, the experiments did not require the consent of the relevant ethics committee for experiments on animals.

Preparation of uterine strips and measurement of their contraction

The uterine strips used in the study were prepared as previously described (Jana et al. 2013, Markiewicz, Jaroszewski 2017). The myometrium was obtained by exfoliating the endometrial layer. Strips of myometrium measuring 3 × 5 mm were taken from the middle part of the uterine horns.

Obtained strips were washed with saline and mounted between two stainless steel hooks in the Schuler organ bath Type 809 (Hugo Sachs Elektronik, March-Hugstetten, Germany) with a resting tension of 10 mN. The strips were suspended in a 5 ml water bath containing Krebs-Ringer solution at 37°C and pH 7.4 with the following composition (mmol l^{-1}): NaCl – 120.3, KCI – 5.9, $CaCl_2$ – 2.5, $MgCl_2$ – 1.2, $NaHCO_3$ – 15.5 and glucose – 11.5. During the experiment, the solution was continuously saturated with a mixture of 95% O_2 and 5% CO_2. An F-30 force transducer type 372 (Hugo Sachs Elektronik) with the bridge connector type 570 was used to measure the contractions of the uterine muscle stripes. Graphical recordings were made on data acquisition equipment using HSE-HA ACAD/W software (Hugo Sachs Elektronik).

Schedule of contractile activity examination

The schedule of treatment of the uterine strips is shown in Figure 1. Recording was started after equilibration for 60-90 min. At the beginning of the study, the strips were incubated with ACh concentrations increasing from 10^{-5} to 10^{-4} M to determine tissue viability and suitability for further study. The strips were then stimulated with BPS or BPAF at concentrations 10^{-13} to 10^{-1} M administered at 15-minute intervals. At the end of each measurement, the uterine strips were washed three times with 15 ml of phosphate buffer. Finally, ACh was administered repeatedly at concentrations 10^{-5} to 10^{-4} M to determine tissue viability. Only results with less than a 20% difference in ACh response before and after treatment were analyzed statistically (Markiewicz et al. 2016).

Pharmacodynamic analysis

The dose-response relationship between pharmacodynamic endpoints (the tension, amplitude and frequency of contractions) and BPAF and BPS concentration was analyzed by nonlinear regression analysis with automatic outlier elimination (Q=10%). All calculations were performed using GraphPad Prism version 9.5.0 (GraphPad Software, San Diego, CA, USA). In the first phase, exploratory dose-response analyses were done using various models. Based on the Akaike information criterion (AIC) and root mean square error (RMSE) value, a model representing the best fit to observed data was selected. Finally, the least squares regression fit with standard Hill slope = -1.0 and log(inhibitor) vs. response (the tension, amplitude and frequency of contractions) model was selected. The following parameters were calculated: E_{max} – the maximal effect value, E_0 – the no effect value (baseline), Span – the dynamic range of the model based on Emax and E0 distance, and $LogIC_{50}$ – the logarithm of the concentration of BPAF and BPS that gives a half-maximal response. The model describes the equation Y = E0 + (Emax-E0)/(1+10(X-LogIC50)), where X represents the logarithm of concentration and Y represents the response.

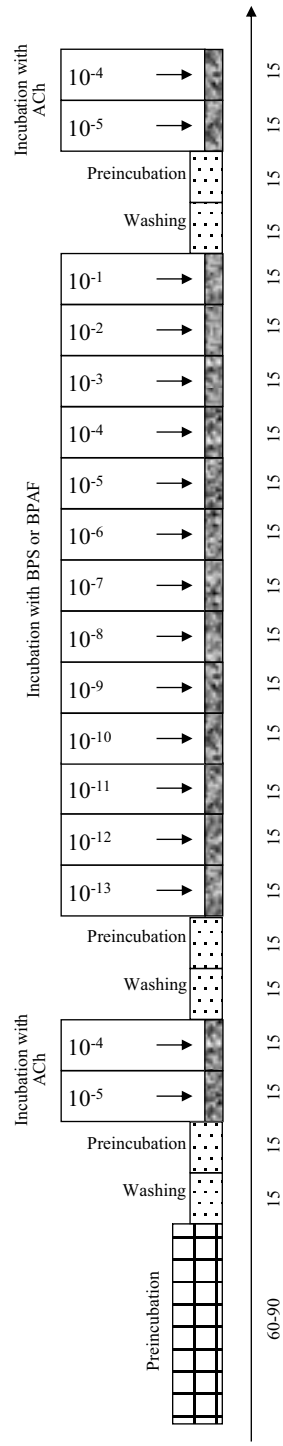

Fig. 1. Scheme presenting the sequence of treatment of the uterine strips: ACh – acetylocholine, BPS – bisphenol S, BPAF – bisphenol AF, concentrations of the applied substances – are given mol 5 mL^{-1}

Statistical analysis

Tension (resting/basal voltage expressed in mN), frequency (number of observed peaks) and amplitude (difference between the minimum and maximum value of a single contraction expressed in mN) of contractions before and after the application of biologically active substances (ACh and BPS or BPAF) were calculated for periods of 15 min, and the values before application were assumed to be 100%. The results calculated for the 15-minute periods after administration of the individual substances at each dose are expressed as a percentage of the tension, frequency and amplitude of the contractions measured during the pre-treatment period. The statistical significance of differences between pre- and post-treatment periods, as well as between the three study groups, was assessed by one-way ANOVA (GraphPad Prism 6.07; GraphPad Software) followed by a Bonferroni multiple comparison test. Three thresholds were assumed as significant differences in the statistics: * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

RESULTS

Effect of BPAF on uterine contractions

BPAF caused a significant decrease in the tension at concentrations of 10^{-6} - 10^{-1} M ($P<0.001$), 10^{-2} - 10^{-1} M ($P<0.05$-$P<0.01$) and 10^{-4} – 10^{-1} M ($P<0.05$-$P<0.001$) in the immature, cyclic and early pregnant group, respectively as compared to the pre-treatment period (Figure 2*a*). At concentrations of 10^{-4} - 10^{-1} M, the tension was significantly lower ($P<0.05$-$P<0.001$) in the immature group as compared to the cyclic group. At concentrations of 10^{-3} - 10^{-1} M, the tension was significantly lower ($P<0.001$) in the immature group as compared to the early pregnant group.

BPAF significantly reduced the amplitude of contractions at concentrations of 10^{-5} - 10^{-1} M ($P<0.05$-$P<0.001$), 10^{-3} - 10^{-1} M ($P<0.01$-$P<0.001$) and 10^{-4} - 10^{-1} M ($P<0.05$-$P<0.001$) in the immature, cyclic and early pregnant groups, respectively, as compared to the pre-treatment period (Figure 2*b*). At concentration 10^{-4} M, the amplitude was significantly lower in the immature group as compared to the cyclic group ($P<0.01$) or the early pregnant group ($P<0.05$). At concentrations of 10^{-2} - 10^{-1} M, the amplitude was significantly lower ($P<0.01$) in the immature group as compared to the cyclic group. At a concentration of 10^{-1} M, the amplitude was significantly lower ($P<0.01$) in the early pregnant group as compared to the cyclic group.

BPAF caused a significant decrease in the frequency of contractions at concentrations of 10^{-11} - 10^{-1} M ($P<0.05$-$P<0.001$), 10^{-1} M ($P<0.001$) and 10^{-7} - 10^{-1} M ($P<0.05$-$P<0.001$) in the immature, cyclic and early pregnant groups, respectively, as compared to the pre-treatment period (Figure 2*c*). The frequency of contractions was significantly lower ($P<0.05$-$P<0.001$) in the imma-

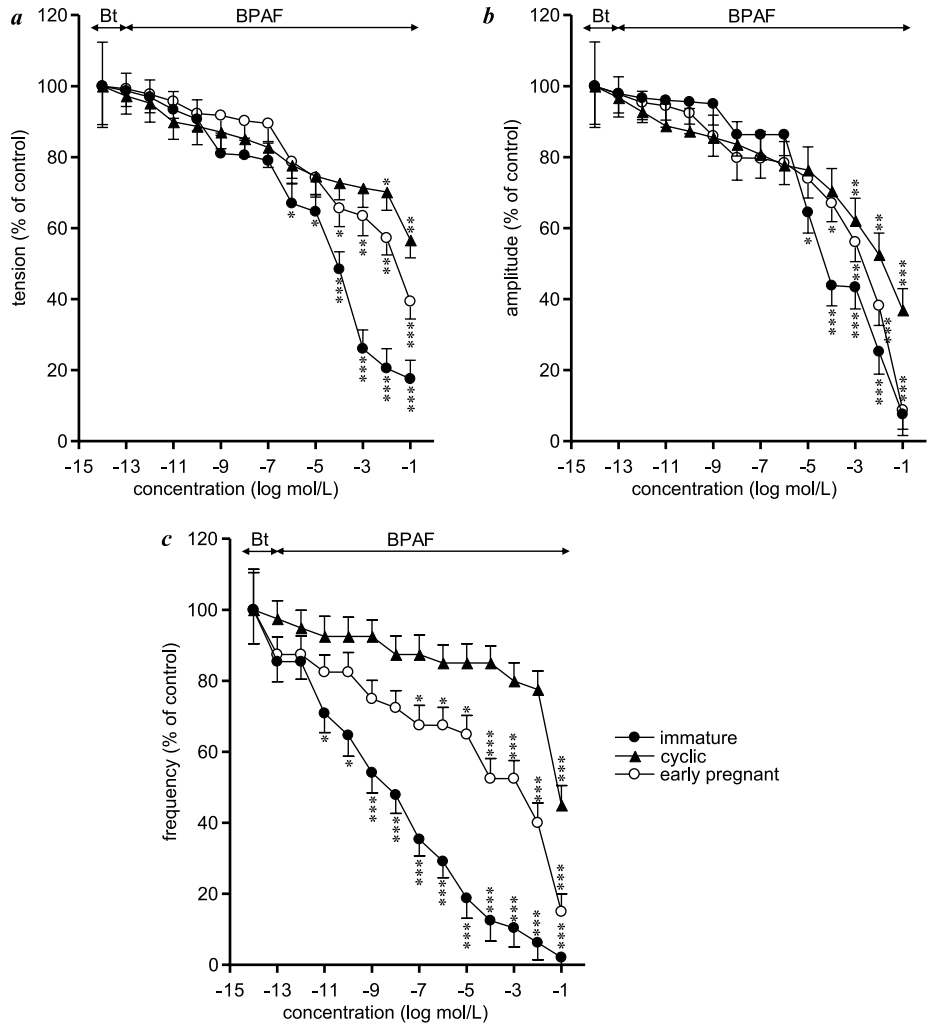

Fig. 2. Influence of bisphenol AF (BPAF) on the tension (*a*), amplitude (*b*) and frequency (*c*) of contractions of the porcine myometrial strips collected from immature, cyclic (on days 12-14 of the estrous cycle) and early pregnant (on days 12-16 of pregnancy) gilts (n=6 in each group). * P<0.05, ** P<0.01, *** P<0.001 compared to the contractile activity before the treatment (Bt)

ture group as compared to the cyclic group at concentrations of 10^{-11} - 10^{-1} M. At concentrations of 10^{-9} - 10^{-1} M, the frequency of contractions was significantly lower (P<0.05-P<0.001) in the immature group as compared to the early pregnant group. At concentrations of 10^{-7} and 10^{-5} - 10^{-1} M, the frequency of contractions was significantly lower (P<0.05-P<0.01) in the early pregnant group as compared to the cyclic group.

Effect of BPS on uterine contractions

BPS significantly reduced the tension at concentrations of 10^{-2} - 10^{-1} M (P<0.05), 10^{-5} - 10^{-1} M (P<0.05-P<0.01) and 10^{-7} - 10^{-1} M (P<0.05-P<0.001) in the immature, cyclic and early pregnant groups, respectively, as compared to the pre-treatment period (Figure 3*a*). At concentrations of 10^{-2} - 10^{-1} M, the tension was significantly lower (P<0.01-P<0.001) in the early pregnant group as compared to the immature and cyclic group.

BPS caused a significant decrease in the amplitude of contractions at concentrations of 10^{-3} - 10^{-1} M (P<0.05-P<0.01) and 10^{-7} - 10^{-1} M (P<0.05-P<0.001) in the immature and the early pregnant group, respectively, with no effect in the cyclic group as compared to the pre-treatment period (Figure 3*b*). At concentrations of 10^{-9} - 10^{-8} M and 10^{-6} - 10^{-1} M the amplitude was significantly lower (P<0.05-P<0.001) in the early pregnant group as compared to the immature group. At concentrations of 10^{-9} - 10^{-1} M, the amplitude was significantly lower (P<0.05-P<0.001) in the early pregnant group as compared to the cyclic group. At a concentration of 10^{-1} M, the amplitude was significantly lower (P<0.01) in the immature group as compared to the cyclic group.

BPS significantly reduced the frequency of contractions at concentrations of 10^{-8} - 10^{-1} M (P<0.05-P<0.001) and 10^{-5} - 10^{-1} M (P<0.05-P<0.001) in the immature and early pregnant group, respectively, with no effect in the cyclic group as compared to the pre-treatment period (Figure 3*c*). The frequency of contractions was significantly lower (P<0.05-P<0.001) in the immature group at concentrations of 10^{-8} - 10^{-1} M as compared to the cyclic group. The frequency of contractions was significantly lower (P<0.05-P<0.001) in the early pregnant group at concentrations of 10^{-5} - 10^{-1} M as compared to the cyclic group.

Pharmacodynamic analysis

A dose-response relationship between pharmacodynamic endpoints (the tension, amplitude and frequency of contractions) and bisphenol concentration was described by the sigmoid dose-response model. The pharmacodynamic and model goodness-of-fit related parameters are presented in Table 1. The presented models well represented the observed data, as indicated by the low AIC values. RMSE shows that residuals are close to the regression line and well describe the model. In all groups, the effect of bisphenol BPS and BPAF on the analyzed pharmacodynamic endpoints was noted. However, the highest dynamic range of the observed effects concerned the amplitude of BPAF. The highest reactivity measured by $LogIC_{50}$ to both bisphenols was noted in relation to cyclic→tension and immature→frequency.

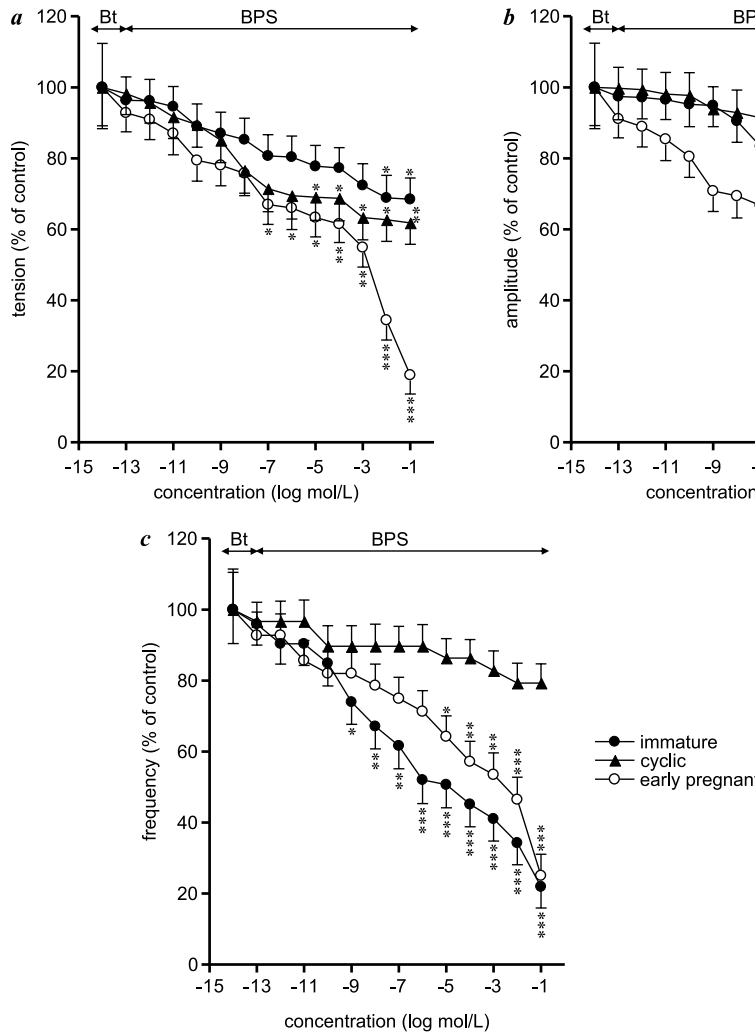

Fig. 3. Influence of bisphenol S (BPS) on the tension (*a*), amplitude (*b*) and frequency (*c*) of contractions of the porcine myometrial strips collected from immature, cyclic (on days 12-14 of the estrous cycle) and early pregnant (on days 12-16 of pregnancy) gilts (n=6 in each group). * P<0.05, ** P<0.01; *** P<0.001 compared to the contractile activity before the treatment *Bt)

DISCUSSION

This study investigated the effect of increasing BPAF and BPS concentrations on the tension as well as the amplitude and frequency of contractions of the porcine myometrium. To the authors' knowledge, this is the first study to assess the effect of these substances on uterine contractile activity

Table 1

Dose-response parameters (arithmetic mean with 90% confidence interval) based on the tension, amplitude and frequency of contractions of the porcine myometrial strips collected from immature, cyclic (on days 12-14 of the estrous cycle) and early pregnant (on days 12-16 of pregnancy) gilts (n=6 in each group) treated with increasing concentrations (10^{-13} to 10^{-1} M) of bisphenol S (BPS) and bisphenol AF (BPAF).

Parameters immature		Tension			Amplitude			Frequency		
		cyclic	pregnant	immature	cyclic	pregnant	immature	cyclic	pregnant	
E_0	BPS	6.861 [6.586 to 7.136]	16.13 [15.55 to 16.72]	8.465 [7.772 to 9.157]	15.04 [13.97 to 16.11]	22.83 [22.32 to 23.34]	6.444 [4.818 to 8.070]	4.954 [4.021 to 5.888]	3.841 [3.762 to 3.919]	2.037 [1.602 to 2.473]
	BPAF	2.791 [1.411 to 4.171]	8.597 [8.010 to 9.185]	6.613 [5.739 to 7.488]	10.91 [8.960 to 12.86]	10.07 [8.540 to 11.61]	1.445 [-1.098 to 3.989]	1.098 [0.4754 to 1.721]	1.977 [0.1391 to 3.814]	2.233 [1.408 to 3.059]
E_{max}	BPS	5.499 [5.259 to 5.739]	11.49 [11.04 to 11.94]	2.514 [0.6692 to 4.358]	22.68 [21.44 to 23.92]	26.12 [25.48 to 26.75]	18.83 [16.89 to 20.78]	10.46 [9.479 to 11.45]	4.308 [4.263 to 4.353]	3.879 [3.588 to 4.169]
	BPAF	9.341 [8.665 to 10.02]	11.25 [10.74 to 11.77]	11.08 [10.48 to 11.68]	30.57 [28.54 to 32.60]	21.20 [19.85 to 22.55]	19.83 [18.16 to 21.50]	5.668 [4.973 to 6.363]	5.958 [5.773 to 6.144]	5.140 [4.691 to 5.589]
Span	BPS	1.362 [1.005 to 1.720]	4.642 [3.925 to 5.360]	5.951 [4.016 to 7.886]	7.636 [6.047 to 9.226]	3.289 [2.495 to 4.083]	12.39 [9.923 to 14.86]	5.510 [4.182 to 6.838]	0.4671 [0.3794 to 0.5548]	1.841 [1.327 to 2.356]
	BPAF	6.550 [5.047 to 8.053]	2.655 [1.889 to 3.420]	4.468 [3.426 to 5.510]	19.66 [16.92 to 22.41]	11.13 [9.171 to 13.08]	18.38 [15.49 to 21.28]	4.570 [3.658 to 5.482]	3.982 [2.165 to 5.798]	2.907 [1.984 to 3.830]
$LogIC_{50}$	BPS	-7.702 [-8.500 to -6.905]	-8.381[-8.848 to -7.914]	-2.886 [-3.590 to -2.181]	-3.599 [-4.205 to -2.993]	-3.843 [-4.530 to -3.156]	-3.710 [-4.286 to -3.134]	-7.311 [-8.050 to -6.573]	-3.520 [-3.993 to -3.047]	-4.895 [-5.668 to -4.122]
	BPAF	-3.669 [-4.259 to -3.079]	-6.322 [-7.203 to -5.441]	-5.065 [-5.715 to -4.415]	-4.872 [-5.250 to -4.494]	-2.740 [-3.195 to -2.285]	-2.232 [-2.587 to -1.877]	-7.580 [-8.200 to -6.960]	-1.457 [-2.065 to -0.8481]	-4.050 [-4.864 to -3.235]
AIC	BPS	-19.39	-1.905	13.84	0.5773	-17.58	11.97	14.93	-44.75	-11.81
	BPAF	12.05	0.4226	6.869	10.38	5.050	12.13	5.047	-18.91	0.9253
RMSE	BPS	0.2994	0.5867	1.075	0.6455	0.3211	1.000	1.121	0.05056	0.4009
	BPAF	1.004	0.6416	0.8222	0.9092	0.7666	1.007	0.7665	0.3051	0.6542

E_{max} – the maximal effect value, E_0 – the no effect value (baseline), Span – the dynamic range of the model based on E_{max} and E_0 distance, $LogIC_{50}$ – the logarithm of the concentration of a bisphenol that gives a half-maximal response, AIC – Akaike information criterion, RMSE – root mean square error

in immature, cyclic and early pregnant animals, i.e. those with different neurohormonal status. The results of the study show that both tested bisphenols exerted a diastolic effect, with differences occurring for both the parameters tested and in individual animal groups. The greatest changes in the tension, amplitude and frequency of contractions following BPAF stimulation were observed in the immature group, while the smallest changes were in the cyclic group. A more varied response was observed following BPS stimulation, as the decrease in tension was the greatest in the early pregnant group while the smallest was in the immature group. As for the amplitude, the greatest decrease was observed in the early pregnant group, with no significant changes observed in the cyclic group. In turn, the reduction in the frequency of contraction was the greatest in the immature group, with no significant changes in the cyclic group. This clearly indicates that the relaxative effect of BPAF and BPS is dependent on the physiological status of the test animals. The obtained results suggest that BPAF has a diastolic effect at lower concentrations than BPS. However, the changes observed for BPAF were the greatest for all the tested parameters (the tension, amplitude, and frequency of contractions) in the immature group, while for BPS, the decrease in the tension and amplitude of contractions was the greatest in the early pregnant group.

Since the available literature provides no data on the effect of BPAF and BPS on uterine contractile activity, it is difficult to compare the current results with studies by other authors. However, the obtained study results clearly show that both tested bisphenols have a diastolic effect similar to that of other compounds in the bisphenol group. The authors' previous study demonstrated that BPA caused the greatest decrease in all tested parameters (the tension, amplitude, and frequency of contractions) in the immature group, while the smallest decrease was in the cyclic group (Zygmuntowicz et al. 2022), similar to what was noted for BPAF in the present study. A relaxative effect of BPA was also observed in the rat (Salleh et al. 2015, Gupta, Deshpande, 2018) and feline (Kabakci et al. 2019) uterus. It seems surprising that inhibition of contractile activity is strongly expressed in the myometrium of sexually immature animals in which the mechanisms regulating the reproductive system function are not fully developed. This phenomenon is difficult to explain because, to date, the mechanism of the diastolic action of bisphenols in the uterus has not been fully understood. It seems that BPS has more potential on the relaxative effect on the early pregnant uterus than those measured by BPAF regarding to tension and amplitude. It was reported that bisphenol treatment in human endometrial Ishikawa cells altered estrogen receptor alpha (ER) signaling and upregulated progesterone receptors (PR) – Fan et al (2021). One might possibly speculate that different BP analogues has relative affinity to the uterus and the second, BPS might have more affinity to uterus than BPAF related to the progesterone, since the progesterone has been already having relaxative effect on the uterus. It is suggested that for BPA, a nitrergic mechanism

is involved in the decrease in the amplitude and frequency of spontaneous uterine contractions in rats (Gupta, Deshpande 2018). However, further studies are needed to identify the specific pathways involved in mediating bisphenols (including BPAF and BPS) effects in the uterus.

When analyzing the effect of the tested bisphenols, it should be noted that their diastolic effect observed in the myometrium of the early pregnant group may have adverse effects on the implantation process. Pope et al. (1982) showed that myometrial contractility increased concomitantly with embryo migration. In addition, the authors' previous study (Markiewicz et al. 2016) showed that the presence of embryos increased contractile activity in the porcine myometrium on days 12-14 of pregnancy, which indicates that the high motor activity in the gravid horn can promote uniform distribution of the multiple embryos in the lumen of the pig uterus.

In conclusion, the results of the experiment show that BPAF and BPS exert a diastolic effect in the porcine uterus, with the effect being dependent on the physiological status of the animals. BPAF exerted the strongest diastolic effect in the uterus taken from sexually immature pigs, while BPS caused the greatest reduction in the tension and amplitude of contractions in the uterus of early pregnant animals, and in the frequency of contractions in the uterus of immature animals.

CONCLUSIONS

In summary, BPS and BPAF at high concentrations significantly reduced porcine uterine smooth muscle contractile activity.

Author contributions

A.Ł. – conceptualization, methodology, formal analysis, investigation, writing – original draft, writing – review & editing, W.M. – conceptualization, formal analysis, funding acquisition, investigation, project administration, writing – review & editing, T.G. -formal analysis, investigation, writing – review & editing, and J.J.J. – conceptualization, formal analysis, investigation, supervision, writing – original draft, writing – review & editing.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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