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Physiological response of cardiac tissue to bisphenol a: alterations in ventricular pressure and contractility

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Submitted 13 April 2015; accepted in final form 14 May 2015


BISPHENOL A (BPA) is widely used in the production of polycarbonate plastics and epoxy resins that are found in a variety of products, including food and beverage containers, aluminum can liners, safety equipment, electronics, water pipes, laminate flooring, thermal printed paper products, dental sealants, and medical devices (15, 24, 47, 55). Consequently, human exposure to BPA can occur routinely via ingestion, inhalation, and dermal contact. Despite the increasing popularity of BPA-free plastics, this chemical additive remains ubiquitous in the environment (21, 22, 56). Indeed, biomonitoring studies have suggested that >90% of the population is exposed to BPA at any given time (16, 54). Lifestyle factors largely influence exposure levels. For example, high urinary BPA levels have been measured in industrial workers (0.02–9 μM) and in patients undergoing medical interventions that use plastic products (4 nM–4 μM) (15, 18, 57). In comparison, serum BPA concentrations range from 1 to 300 nM (2, 8, 14, 16, 24, 27, 32, 40, 49, 50, 61). For in vitro experimental studies, ≤10−7 M has been previously defined as “low-dose” BPA exposure (58).

Human epidemiological studies have recently reported correlations between high urinary BPA concentrations and an increased prevalence of cardiovascular disorders, including hypertension (1, 5, 6, 52), atherosclerosis (31, 34–37, 51), angina and myocardial infarction (31), and reduced heart rate variability (6). Even with this correlative insight, relatively little is known about the physiological response of the cardiovascular system when exposed to BPA. We have previously reported that acute BPA exposure (10−7–10−4 M) alters cardiac electrophysiology, and others have shown a link between BPA exposure and altered cardiac Ca2+ handling (17, 20, 59). Both slowing of cardiac impulse propagation and altered Ca2+ cycling could detrimentally impact mechanical function, resulting in reduced developed pressure and contractility. The goal of the present study was to systematically assess the direct effect of BPA on whole heart contractile function. The physiological response to BPA exposure was compared between male and female hearts, and adaptations to high heart rates were also identified.

MATERIALS AND METHODS

Animals. All animal protocols were approved by the George Washington University’s Animal Care and Use Committee and followed the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. Experiments were conducted using excised per-
fused hearts from female (n = 23) and male (n = 5) Sprague-Dawley rats (2–3 mo of age, body weight: 200–250 g). Rats were purchased from Hilltop Lab Animals (Scottsdale, PA). Two animals were housed per cage at The George Washington University animal care facility under standard environmental conditions [12:12-h light-dark cycle, 64–79°C, 30–70% humidity, and corn cob bedding (Harlan Laboratories, Indianapolis, IN)] with free access to food (2018 Teklad Global rodent chow, Harlan Laboratories) and carbon-filtered tap water.

For cell experiments, primary cardiomyocytes were isolated from 1-day-old Sprague-Dawley rats (Hilltop Lab Animals) by an enzymatic digestion protocol, as previously described (3, 46). Experiments were performed after cells formed a confluent synchronously beating cardiac network (2–3 days after being plated, n = 18).

Excised heart preparation and contractility experiments. Rats were anesthetized with 4% isoflurane and an intraperitoneal injection of Telazol (50 mg/kg). After cessation of pain reflexes, hearts were quickly excised, and the aorta was cannulated and perfused at constant pressure (70 mmHg) and constant temperature (37°C) with modified Krebs-Henseleit solution (23), as previously described (38). Hearts were superfused in a temperature-controlled chamber for electrical and mechanical measurements (Fig. 1). Three electrodes were placed in the superfusate, and the ECG was continuously measured throughout each experiment. A latex balloon (size 4) filled with perfusate was inserted into the left ventricle (LV). Uncompressed balloon pressure (diastolic preload pressure) was maintained at 7–10 mmHg. Isoluminometric LV developed pressure (LVDP) was measured as changes in balloon pressure using a pressure transducer (Harvard Apparatus) connected to a bridge amplifier. The ECG and LV pressure were acquired using a PowerLab unit with LabChart software (AD Instruments). LVDP was calculated as the difference between maximum systolic and minimum diastolic pressure. Contractility was calculated as the rate of pressure development during systole ($dP/dt_{max}$) and the rate of relaxation during diastole ($dP/dt_{min}$). A bipolar stimulating electrode was placed on the ventricular epicardial surface to control heart rate during experimental protocols.

General protocol. BPA (>99% purity, Sigma-Aldrich) was dissolved in 100% ethanol to provide a 500 mM stock solution. During the experiments, aliquots of stock solution were added to the perfusate media to obtain final concentrations of 0 M (control) to $10^{-4}$ M BPA, with 10% ethanol concentrations ranging from 0.01% for the control and 0.02% for $10^{-4}$ M BPA. The highest ethanol concentration (0.02%) was confirmed to have no effect on measured variables, as detailed in the RESULTS. BPA contamination from external sources was minimized by preparing solutions in glass bottles and using BPA-free Tygon and C-Flex Tubing (Cole Parmer).

Each experiment was completed within 60 ± 24 min (mean ± SD) after heart excision. On average, three BPA aliquots were sequentially added to the perfusate media (i.e., animal 1: control $\rightarrow 10^{-9}$ M BPA $\rightarrow 10^{-8}$ M BPA $\rightarrow 10^{-7}$ M BPA) with hearts exposed to each concentration for 15 min (both control and treated; Fig. 1A). The LV apex was paced to account for slight variations in sinus rate between animals and experiments. There were three pacing rates (5, 6.5, and 9 Hz) with ±5 s at each pacing rate and 2-mA pulses (5-ms pulse duration). There was a 30-s interval between each pacing sequence. This approach also revealed heart rate-dependent changes that indicated how BPA may alter the response of the heart to acute changes in workload. In each experiment, measurements were normalized to the control to account for variability between heart preparations. Data are reported as percent change from control measurements, unless otherwise noted.

Whole heart Ca$^{2+}$ imaging. The effect of BPA on intracellular Ca$^{2+}$ cycling was measured in a separate set of experiments using a Ca$^{2+}$-sensitive dye (rhod-2 AM). Hearts were excised and perfused as described for the contractility experiments. Rhod-2 AM (15 μM, Molecular Probes) was administered as a bolus injection to the aorta and recirculated through the myocardium for 10 min. To prevent motion artifacts in Ca$^{2+}$ signals, contraction was inhibited by admin-

Fig. 1. Experimental protocol. A: ECG and left ventricular (LV) pressure (LVDP) signals were acquired continuously throughout the study during both intrinsic sinus rhythm and throughout pacing protocols. The average experiment was composed of three treatments [control (Ctrl), bisphenol A (BPA) protocol 1 (BPA1), BPA protocol 2 (BPA2), and BPA protocol 3 (BPA3)] over 60 ± 24 min. B: excised rat heart with three-lead ECG and latex balloon inserted into the LV. LA, left atrium; RA, right atrium. C and D: heart rate (HR) and pressure signals remained stable in the presence of vehicle (Veh) control (ethanol) throughout the 90-min experiment. n = 4 female rat hearts. LVDP, LV developed pressure; ns, not significant relative to the corresponding control (P > 0.05).

istering the actomyosin inhibitor blebbistatin to the perfusate at a circulating concentration of 10 μM. Rhod-2 AM was excited by illuminating the epicardium with light from a LED spotlight (530 nm, Mightex, Pleasanton, CA) that was filtered at 545/20 nm (Chroma Technology, Bellows Falls, VT). Light emitted from the epicardium was band-pass filtered at 605/35 nm (Chroma Technology) and imaged at 600 frames/s using an iXon DV860 EMCCD camera (Andor Technology, Belfast, UK).
**Neonatal cardiomyocyte Ca\(^{2+}\) imaging.** Neonatal rat cardiomyocytes were cultured as previously described (3, 46). Monolayers were loaded with 10 \(\mu\)M fluo-4 AM for 30 min at room temperature and then washed in dye-free Tyrode salt solution (Sigma-Aldrich). Monolayers were then exposed to either 0.01% ethanol (control) or \(10^{-4}\) M BPA in Tyrode solution (Sigma-Aldrich) for 15 min at room temperature. Cardiac monolayers were then paced using a bipolar electrode (Harvard Apparatus, Holliston, MA) to which monophasic 5-ms pacing pulses were applied at 0.2 Hz. Pace-induced Ca\(^{2+}\) transient recordings were acquired at 36 frames/s using a Zeiss LSM 510 confocal imaging system (488-nm excitation, 505- to 530-nm emission).

**Statistical analysis.** Data were normalized as a percentage of baseline during perfusion with control media (before treatment with BPA) at a 5-Hz pacing rate and are presented as means ± SE, unless otherwise noted. The lowest dose resulting in a statistically significant effect was determined by one-way or two-way ANOVA followed by individual t-tests, as previously described (13, 47). All results were computed from \(n \geq 4\) independent experiments (animals) at each dose, with statistical significance determined at \(P \leq 0.05\).

**RESULTS**

Preliminary heart experiments (\(n = 4\) female rats) were performed without BPA to confirm that electromechanical function during perfusion with control media or vehicle control media (0.02% ethanol) was maintained. Heart rate, LVDP, and dP/dt max remained within 92 ± 6%, 97 ± 8%, and 108 ± 5%, respectively, of initial values after 90 min of perfusion with control and vehicle control media (Fig. 1, C and D).

**Effect of BPA during sinus rhythm.** In accordance with our previously published study (45), sinus heart rate remained stable after administration of low doses of BPA. Significant heart rate reductions were only observed at BPA doses of >\(10^{-5}\) M (59 ± 9%, \(P < 0.0001\); Fig. 2, A and B). Heart block was observed in 56% of hearts exposed to the highest tested concentration (\(10^{-4}\) M BPA, 15-min exposure), which was identified in ECG signals by the loss of 1:1 coupling between the P wave and QRS complex (Fig. 2A, right). Despite similar heart rates between all other treatment groups, significant reductions in LVDP (77 ± 6%, \(P < 0.005\)) and dP/dt max (85 ± 6%, \(P < 0.05\)) were observed beginning at \(10^{-7}\) and \(10^{-6}\) M BPA, respectively (Fig. 2B). This finding could be attributed to slowed conduction (45) and/or alterations in intracellular Ca\(^{2+}\) handling (11, 25).

**Effect of BPA on pressure development.** Cardiac contractility and pressure development are dependent on heart rate, whereby contractility increases at faster heart rates, as predicted by the Bowditch effect (11, 30). At pacing rates similar to the resting heart rate of a rat (5–6.5 Hz), BPA-induced reductions of LVDP were observed beginning at \(10^{-7}\) M BPA (Fig. 3B). Yet, at a faster rates corresponding to the heart rate of a rat during exercise or stress (9 Hz), reductions in LVDP were more pronounced with statistically significant reductions in LVDP measured at the lowest BPA dose (\(10^{-9}\) M, 88 ± 4%, \(P < 0.005\)).

Postrest potentiation is a phenomenon by which an increase in developed pressure is observed after a brief period of rest (Fig. 3A). During the rest period, Ca\(^{2+}\) reuptake into the sarcoplasmic reticulum (SR) is increased, and, as such, the postrest contraction is potentiated due to increased SR Ca\(^{2+}\) load (44). We detected pronounced LVDP postrest potentiation in control hearts (Fig. 3C); however, LVDP postrest potentia-

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**Fig. 2.** BPA effects during sinus rhythm. A: ECG and LVDP signals acquired during control perfusion and after treatment with BPA (15 min). B: HR slowing was observed only at concentrations of >\(10^{-5}\) M BPA, which frequently resulted in atrioventricular block. Reductions in LVDP and dP/dt max were observed beginning at \(10^{-7}\) and \(10^{-6}\) M BPA, respectively. \(n = 22\) female rat hearts. *\(P \leq 0.05\) relative to the corresponding control.
tion was significantly reduced (−13% of control) at the lowest BPA concentration tested (10⁻⁹ M, 9-Hz pacing, P < 0.05).

**BPA exposure reduces contractility and lusitropy.** Maximum rates of force development (contractility, dP/dt max) and relaxation (lusitropy, dP/dt min) during pacing and after a period of rest were measured. The effects of BPA on dP/dt max were significantly dependent on BPA concentration and pacing rate. The rate of pressure development decreased by 12% at 5-Hz pacing and 44% at 9-Hz pacing, relative to control, in hearts perfused with 10⁻⁵ M BPA (Fig. 4B). Alterations in contractility were intensified in postrest measurements, in which dP/dt max decreased by 17%, relative to control, at 9-Hz pacing after exposure to the lowest BPA dose (10⁻⁹ M BPA; Fig. 4C). dP/dt min was minimally affected by BPA exposure, with significant decreases only observed at the highest dose tested (10⁻⁴ M, P < 0.05; Fig. 4D). Alterations in lusitropy became more prominent in postrest pressure measurements; dP/dt min decreased by 20% at 5-Hz pacing and 43% at 9-Hz pacing in hearts perfused with 10⁻⁷ M BPA compared with the corresponding control hearts. Significant effects on contractility but minimal effects on lusitropy may indicate differences in systolic and diastolic Ca²⁺ handling whereby SR load and synchronized Ca²⁺ release are predominately affected by BPA exposure compared with the rate of Ca²⁺ reuptake.

**BPA exposure affects cardiac physiology in both male and female rats.** Previous reports have indicated that the effect of BPA on cardiac physiology is sex specific, with the mechanism largely attributed to the interaction between BPA and estrogen receptors in females (59). Importantly, we detected reductions in heart rate, LVDP, and dP/dt max in both male and female hearts in the presence of increasing concentrations of BPA. Although heart rate slowing was observed at concentrations of >10⁻⁵ M BPA for both sexes, female hearts slowed to a greater extent (−45%) versus male hearts (−15%, P < 0.05; Fig. 5A). A significant reduction in LVDP was also observed at lower BPA concentrations in female versus male hearts (10⁻⁸, 10⁻⁷ M, P < 0.05; Fig. 5B), but no significant sex differences were observed in dP/dt max values (Fig. 5C).

**BPA exposure alters intracellular Ca²⁺ handling.** Ca²⁺ cycling is an important mediator of cardiac contraction and relaxation. At faster heart rates, an increasing amount of Ca²⁺ is released from the SR with each contraction due to increased SR loading (12, 28). In hearts exposed to BPA (10⁻⁵ M), fast heart rates (9 Hz) did not elicit the same adaptive change in diastolic and systolic Ca²⁺ cycling compared with control hearts (Fig. 6, A and B). Similar effects were observed in neonatal cardiomyocyte monolayers. BPA-treated monolayers displayed reduced Ca²⁺ transient amplitudes (−37% for 10⁻⁴ M BPA, P = 0.0001) and prolonged Ca²⁺ transient upstroke time (+133% for 10⁻⁴ M BPA, P = 0.001; Fig. 7, B and C), suggesting reduced SR Ca²⁺ loading and slower SR Ca²⁺ release.

**DISCUSSION**

We have previously reported that BPA exposure significantly impacts cardiac electrical function (45). BPA exposure delayed atrioventricular conduction, slowed ventricular conduction velocity, and prolonged action potential duration in a dose-dependent manner. If similar physiological responses were to occur in vivo, exposure to this common environmental chemical could be problematic for individuals with preexisting cardiac conduction abnormalities (i.e., bundle branch block, bradycardia, or arrhythmia). Other recent work has also high-

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Fig. 3. BPA exposure reduces developed pressure. A: LVSP signals in response to external pacing (denoted by ↓) are potentiated (denoted by ↑) after a period of rest ("postrest"). B and C: reductions in LVDP in response to BPA dose and pacing frequency (B) and in postrest potentiated signals (C). n = 23 female rat hearts. *P ≤ 0.05 relative to the corresponding control.

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[AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00272.2015 • www.ajpheart.org]
lighted the effect of BPA on cardiac electrophysiology, including increased incidence of spontaneous aftercontractions (59) and inhibition of Na\(^+\) and Ca\(^{2+}\) channel currents (17, 39). Abnormalities in electrical conduction and excitation-contraction coupling can negatively affect pressure development, sometimes dramatically, especially during heart failure (53). With this in mind, our goal was to systematically measure the effect of BPA exposure on cardiac contractile performance.

In the present study, we observed a dose-response relationship between acute (15 min) BPA exposure and cardiac mechanical function. This was observed during sinus rhythm and when pacing the apex of the heart. Despite stable heart rates, significant reductions in LVDP and dP/dt during sinus rhythm were observed at BPA concentrations of 10\(^{-7}\) and 10\(^{-6}\) M, respectively. Pacing the apex, which circumvents the cardiac conduction system, produced similar results. At rates corresponding to the resting heart rate of a rat (5–6.5 Hz) (4), significant reductions in LVDP were measured beginning at 10\(^{-9}\) M, and significant reductions in dP/dt\(_{\text{max}}\) were measured beginning at 10\(^{-7}\) M BPA. Despite significant decreases in contractility, relaxation was unaffected except at very high BPA concentrations (10\(^{-4}\) M) that exceed clinical relevance.

Our results in excised whole hearts are similar to those of Pant et al. in isolated atrial preparations (42). They observed significant slowing of the rate of atrial contraction at concentrations exceeding 10\(^{-5}\) M BPA. They also observed reductions in contractile force beginning at 10\(^{-7}\) M BPA (−25% vs. control). The authors identified alterations in nitric oxide/
cGMP signaling as a mechanism for the effect of BPA on atrial contractility. This is consistent with previous work showing that nitric oxide synthases influence Ca\textsuperscript{2+} handling at both the L-type Ca\textsuperscript{2+} channel and SR levels (7, 29).

In our study, maximal BPA effects on cardiac contractile performance were detected at elevated heart rates and in postrest contractions. The myocardium exhibits a force-frequency response (FFR) whereby developed pressure increases with stimulation frequency (26). As heart rate is increased, the activity of sarco(endo)plasmic reticulum Ca\textsuperscript{2+} ATPase (SERCA) increases to pump more Ca\textsuperscript{2+} into the SR. As a result, more Ca\textsuperscript{2+} is released from the SR with each subsequent contraction until a new equilibrium is reached. Then, after a brief period of rest, contraction amplitude is significantly potentiated in the first “postrest” beat (Fig. 3A) (44). This beat can serve as an indicator of SR Ca\textsuperscript{2+} load or capacity. Our results show that postrest potentiated contractions, after a 9-Hz pacing protocol, have significantly reduced LVDP and significant reductions in both dP/dt\textsubscript{max} and dP/dt\textsubscript{min} beginning at nanomolar BPA concentrations. This is important because these concentrations (≤10$^{-7}$ M BPA) have been accepted as a low dose for in vitro studies (58).

We also examined the association between BPA exposure and excitation-contraction coupling by imaging Ca\textsuperscript{2+} transients from the epicardial surface of excised rat hearts. Hearts perfused with BPA exhibited a flattened FFR, with fewer changes in systolic and diastolic Ca\textsuperscript{2+} over time, compared with control perfusion (Fig. 6). A flat or negative FFR has been reported in heart failure models and can indicate impaired contractile reserve and abnormal exercise tolerance (48). In such pathological cases, alterations in the FFR have been largely attributed to increased Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger and/or reduced SERCA activity (41, 48). Although these mechanistic alterations develop slowly in disease states, it is possible for changes in SERCA activity or channel current to occur quickly. For example, PKA-Ca\textsuperscript{2+}/calmodulin-dependent kinase II signaling can modify the phosphorylation state of phospholamban, which can immediately affect SERCA Ca\textsuperscript{2+} reuptake (9, 10, 19).

To gain more insight into the Ca\textsuperscript{2+} dynamics of BPA-perfused hearts, we also imaged Ca\textsuperscript{2+} transients from cardiomyocyte monolayers. Consistent with our whole heart experiments, in BPA-treated monolayers, we measured reduced Ca\textsuperscript{2+} transient amplitudes and prolonged Ca\textsuperscript{2+} transient upstrokes. Taken together, our results suggest that BPA alters cardiac Ca\textsuperscript{2+} cycling and may reduce SR Ca\textsuperscript{2+} load and slow the release of SR Ca\textsuperscript{2+}.

Although others have reported the effects of BPA on cardiac Ca\textsuperscript{2+} handling, the mechanism remains unclear. Yan et al. (59) reported an increase in the incidence of spontaneous aftercontractions in female adult rat ventricular myocytes exposed to BPA. This effect was linked to downstream estrogen receptor signaling, which involved PKA-Ca\textsuperscript{2+}/calmodulin-dependent kinase II signaling, ultimately resulting in increased SR Ca\textsuperscript{2+} load and increased SR leak via phosphorylation of phospholamban and ryanodine receptors, respectively (20). The same group has also shown that in adult rat ventricular myocytes from female hearts, BPA inhibits L-type Ca\textsuperscript{2+} channels in a
dose-dependent manner (33). Yet, some of these effects were transient and disappeared after ≥5 min of exposure to BPA. In comparison, a chronic in vivo BPA exposure study (43) has revealed significant phospholamban dephosphorylation in female rats, which would likely inhibit SERCA activity and decrease SR load and Ca\(^{2+}\) transient amplitude. The results of our present work support these previous observations.

Previous studies have shown that BPA exposure elicits sex-specific effects on cardiac function that are mediated via estrogen receptor signaling. Using an excised whole heart model, we found differences in sinus heart rate, LVDP, and dP/dr in both male and female hearts. Although a greater reduction in LVDP was measured at intermediate BPA concentrations (10\(^{-8}\)-10\(^{-5}\) M) in female hearts, male hearts were not completely safeguarded from the effects of BPA. Additional studies are necessary to pinpoint the influence of BPA on cardiac mechanical performance in cellular models versus three-dimensional cardiac tissue.

In conclusion, we measured a dose-dependent decline in cardiac performance upon acute exposure to BPA. BPA-induced alterations in LV pressure, contractility, and lusitropy were maximal at elevated heart rates and in postrest contractions. If similar physiological responses were to occur in vivo, exposure to this common environmental chemical may be a risk factor for individuals predisposed to contraction abnormalities (i.e., heart failure, age-associated fibrosis, or patients with myocardial infarction). Future studies are necessary to determine whether the observed effects are specific to species, sex, and the age of an individual and whether these cardiac effects are further exacerbated by prolonged exposure to BPA.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Dr. Luther Swift, Angel Moreno, and Rabia Idrees for assistance with the Langendorff perfusion preparations.

GRANTS

This work was supported by National Institutes of Health Grants K99-ES-023477, R01-HL-095828, and R21-HL-122882.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: N.G.P., conception and design of research; N.G.P., D.B., A.C., and R.J. performed experiments; N.G.P., D.B., and A.C. analyzed data; N.G.P., N.S., and M.W.K. interpreted results of experiments; N.G.P. prepared figures; N.G.P. drafted manuscript; N.G.P., N.S., and M.W.K. edited and revised manuscript; N.G.P., D.B., A.C., R.J., N.S., and M.W.K. approved final version of manuscript.

Fig. 7. BPA alters Ca\(^{2+}\) handling in isolated cardiomyocytes. A: confluent layer of neonatal rat cardiomyocytes. B: example of CaTs recorded from cardiomyocytes in cell layers. C: BPA exposure reduced CaT amplitude and slowed CaT upstroke time. n = 18 cardiomyocyte layers. *P ≤ 0.05 relative to the corresponding control.

Fig. 6. BPA alters Ca\(^{2+}\) handling in whole hearts. A: Ca\(^{2+}\) transient (CaT) recording from optically mapped hearts (9-Hz pacing rate). B: control hearts displayed a robust adaptation to fast HRs by increasing systolic and diastolic Ca\(^{2+}\) cycling (ΔF/Δt; force-frequency relationship). BPA-treated hearts displayed a flattened response. n = 4 female rat hearts. *P ≤ 0.05 relative to the corresponding control.


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