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Bisphenol A Exposure and Cardiac Electrical Conduction in Excised Rat Hearts

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Running title: Effect of BPA on cardiac electrical conduction

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Abstract

Background: Bisphenol A (BPA) is used to produce polycarbonate plastics and epoxy resins that are widely used in everyday products, such as food and beverage containers, toys and medical devices. Human biomonitoring studies have suggested that a large proportion of the population may be exposed to BPA. Recent epidemiological studies have reported correlations between increased BPA urinary concentrations and cardiovascular disease; yet the direct effects of BPA on the heart are unknown.

Objectives: The goal of our studies was to measure BPA's effect (0.1-100 μM) on cardiac impulse propagation *ex vivo*, using excised whole hearts from adult rats.

Methods: We measured atrial and ventricular activation times during sinus and paced rhythms using epicardial electrodes and optical mapping of transmembrane potential. Atrioventricular activation intervals and epicardial conduction velocities were computed using recorded activation times.

Results: Cardiac BPA exposure resulted in prolonged PR segment and decreased epicardial conduction velocity (0.1 – 100 μM), prolonged action potential duration (1 – 100 μM) and delayed atrioventricular conduction (10 – 100 μM). Importantly, these effects were observed after acute exposure (≤ 15 min), underscoring the potential detrimental effects of continuous BPA exposure. The highest BPA concentration used (100 μM) resulted in prolonged QRS intervals, dropped ventricular beats and eventually resulted in complete heart block.

Conclusions: Our results show that acute BPA exposure slows electrical conduction in excised hearts from female rats. These findings emphasize the importance of examining BPA's effect on heart electrophysiology and determining whether chronic *in vivo* exposure can cause/exacerbate

conduction abnormalities in patients with pre-existing heart conditions and other high-risk populations.

Introduction

Bisphenol A (BPA) is one of the most widely used chemicals worldwide, with over 8 million pounds produced each year (Vandenberg et al. 2010). BPA is a component of polycarbonate plastics and epoxy resins, which are used in many plastic consumer products including food and drink containers, water pipes, thermal paper and paper products (receipts, paper towels, etc.), toys, safety equipment, electronics, dental monomers, medical equipment and tubing. It has been reported that BPA can leach from these products normal conditions (Vandenberg et al. 2007). Despite the increasing popularity of BPA-free plastics, BPA is still found in many consumer products. Indeed, human biomonitoring studies suggest that a large proportion of the population may be exposed to BPA, including both children and adults (Calafat et al. 2005; Calafat et al. 2008). BPA exposure rates range dramatically depending on lifestyle factors, with neonates in intensive care units (4.4 nM – 4 μM) and industrial workers (0.024 – 8.5 μM) having overall higher urinary BPA concentrations (Calafat et al. 2009; Wang et al. 2012). Human serum BPA levels are actively debated, with estimates ranging from 0.001 – 0.3 μM for adults (Kaddar et al. 2009; Lee et al. 2008; Padmanabhan et al. 2008; Schonfelder et al. 2002; Vandenberg et al. 2010). Patients undergoing multiple medical interventions – such as neonates in intensive care – may have even higher levels of BPA in the blood. However, BPA serum levels have not been measured in this patient population.

There is evidence that increased exposure to endocrine-disruptors, such as BPA, might contribute to the onset and progression of disease (Braun and Hauser 2011; Chapin et al. 2008; Diamanti-Kandarakis et al. 2009; Richter et al. 2007; Vandenberg et al. 2007; vom Saal et al. 2007). Recent epidemiological studies have shown an association between BPA exposure and

cardiovascular disease. Higher BPA urine concentrations have been associated with an increased risk of coronary artery disease (Melzer et al. 2010; Melzer et al. 2012), hypertension (Shankar et al. 2012), carotid atherosclerosis (Lind and Lind 2011), angina and myocardial infarction (Lang et al. 2008), and a decrease in heart rate variability (Bae et al. 2012). Although the latter is primarily attributed to neuronal influences, decreased heart rate variability can also indicate alterations in ion channel currents that drive pacemaker depolarization (Papaioannou et al. 2013). In addition, *in vitro* experimental studies have shown that higher concentrations of BPA exposure modifies heart rate in isolated atrial preparations (Pant et al. 2011). Both of these observations suggest alterations of ionic currents in nodal cells, which can influence nodal and bundle branch conduction. Moreover, if BPA induces ion channel alterations in atrial tissue, exposure will likely affect ion channels in other tissue compartments. Importantly, the effect of BPA on heart electrophysiological function has not been examined. We aimed to systematically study the effect of BPA on cardiac conduction by conducting controlled experiments on excised whole rat hearts. These studies provide new measurements for important electrophysiological parameters, including AV conduction and ventricular conduction velocity.

Methods

Animals

Experiments were conducted using excised hearts from adult female Sprague-Dawley rats (n = 12, 200-300 g, 2-3 months old), purchased from Hilltop Lab Animals (Scottsdale, PA). Studies were limited to females, as previous reports have claimed that BPA's cardiac effects are sex specific due to its estrogenic properties (Belcher et al. 2012; Yan et al. 2011). To account for the possible effects of estrous cyclicity on cardiac electrophysiology, each animal served as its own

control with all BPA measurements computed as a percent change from control (no BPA). Animals were housed in The George Washington University (GWU) animal care facility under standard environmental conditions (12:12 h light: dark cycle, 64-79°C, 30-70% humidity, corn cob bedding (Harlan Laboratories, Indianapolis, IN) with free access to food (2018 rodent chow (Harlan Laboratories, Indianapolis, IN)) and carbon-filtered tap water. Animals were housed in groups of 2-3 animals per cage for 2 weeks prior to experimentation. All animals were treated humanely and with regard for alleviation of suffering. All procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at GWU.

Excised heart preparation

Hearts were excised and Langendorff-perfused with a modified Krebs Henseleit buffer (Gillis et al. 1996), as previously described (Mercader et al. 2012). Hearts were placed in a temperature-controlled chamber (Figure 1A) for electrical measurements. The perfusate was supplemented with 10 μM blebbistatin (Sigma Aldrich, St. Louis, MO) to reduce motion.

General protocol

99+% purity BPA (Sigma Aldrich, St. Louis, MO) was prepared fresh for each experiment. A stock solution was prepared in 100% ethanol, and final dilutions were made directly in perfusate media with a total ethanol concentration of 0.01-0.02% (no BPA (control) – 100 μM BPA, respectively). Control perfusate contained 0.01% ethanol and no BPA. Precautions were maintained throughout the study to prevent BPA contamination from external sources (i.e., solutions mixed and stored in glass bottles, BPA-free Tygon and C-Flex tubing [Cole Parmer, Vernon Hills, IL] used for Langendorff-perfusion). However, the perfusate media was not analyzed for BPA contamination.

Excised hearts maintained electrophysiological function for > 3 hours with control media perfusion (no BPA). Each study began midday and was completed in 1-2 hr; steps included: 1) Perfuse with control media. 2) Position electrodes and electrocardiogram (ECG) leads (5-10 min). 3) Record ECG and atrioventricular (AV) conduction signals during sinus rhythm (15 min equilibration period). 4) Implement pacing protocol and acquire optical signals (see below). 5) Dilute BPA directly in perfusate media to achieve the appropriate final concentration. 6) Record ECG and AV conduction signals during sinus rhythm (15 min). 7) Implement pacing protocol and acquire optical signals. 8) Repeat steps 5-7 for additional BPA concentrations. For each preparation, the heart was exposed to an average of three BPA concentrations added incrementally to the perfusate media (i.e., Animal #1: Control → 0.1 → 1 → 10 μM BPA, Animal #2: Control → 1 → 10 → 25 μM BPA, 15 min exposure each). Each animal served as its own control with measurements normalized to control perfusion in the same heart and expressed as a percent change. This allowed us to account for variations in electrode position for each experiment, and to reduce any influence of estrous cyclicity on cardiac conduction measurements.

The epicardial pacing protocol consisted of pacing the heart with 2 mA pulses (5 msec duration) for ≥ 5 sec at 5-15 Hz pacing frequencies (incremented stepwise by 1 Hz). There was a 30 sec interval after each pacing sequence before beginning the next.

Electrical measurements

AV conduction was measured as the time difference between activation recorded by each electrode (right atria and ventricular apex) during sinus rhythm (Figure 1A,B), as determined by the maximum signal derivative. For dose-response analysis, AV delay was measured during

sinus rhythm after 15 min exposure to control or BPA-containing media. Sinus heart rate remained stable during control perfusion (4.1 ± 1 Hz), but slowed after exposure to 50–100 μM BPA (3.6 ± 1 Hz, 3.3 ± 1 Hz, respectively). However, this slowing did not reach significance until the onset of AV block (described below). All electrical signals were amplified (Dagan EX4-400 differential amplifier; Dagan Corporation, Minneapolis, MN) and recorded using a PowerLab data acquisition system (ADInstruments, Colorado Springs, CO). Electrical signals, including AV delay and ECG segments, were analyzed using LabChart software (ADInstruments, Colorado Springs, CO; $n \geq 4$ signals per concentration for each independent experiment).

Epicardial conduction velocity (CV) measurements

The effect of BPA on CV was studied using epicardial electrodes and optical mapping. CV is rate-dependent and decreases at high pacing frequencies, such rate dependent changes are referred to as CV restitution (Kleber and Rudy 2004; Qu et al. 2004). Therefore, a pacing protocol was implemented that gradually increased pacing frequencies (5–15 Hz) to reveal the effect of BPA on CV. Epicardial conduction time (sec) was computed as the difference between the onset of the pacing pulse and the activation time measured from the apical recording electrode (Figure 1A). Epicardial CV (cm/sec) was computed by dividing conduction time by electrode separation distance. CVs were calculated for each pacing frequency (5–15 Hz, see pacing protocol). Measurements were normalized to corresponding controls at 5 Hz pacing frequency (near intrinsic rate). Electrical signals were analyzed using LabChart software ($n \geq 4$ signals per concentration for each independent experiment).

Optical mapping of wavefront propagation

Optical mapping is a powerful technique for studying cardiac electrical conduction, which has been used for more than a decade to reveal mechanisms of arrhythmia (Laughner et al. 2012). We used optical mapping of a potentiometric dye (RH237, Life Technologies, Carlsbad, CA) to study alterations in electrical conduction using techniques developed in our laboratory (Asfour et al. 2011; Kay et al. 2006; Swift et al. 2008). RH237 dye kinetics are faster than changes in transmembrane potential during an action potential (Rosenbaum and Jalife 2001), and RH237 has been used successfully in our laboratory to study cardiac electrical activity. RH237 was administered to the aorta (5 mL of a 10 μM solution) before beginning a pacing protocol (described above). To record optical action potentials, the epicardium was illuminated using an LED spotlight (530/35 nM, Mightex, Pleasanton, CA). RH237 fluorescence was longpass filtered at 680nm and imaged using a CCD camera (Ixon DV860, Andor Technology, Belfast, UK), as previously described (Mercader et al. 2012). At each pacing frequency, optical signals were acquired and mapping data was quickly viewed to verify continuous elliptical wavefront propagation originating from the pacing electrode. Activation times were identified for each pixel and local CVs were computed (Bayly et al. 1998) to generate isochronal maps. An average CV was computed by averaging the values of individual CV vectors across the epicardial field of view. Optical action potentials were analyzed using custom Matlab software (MathWorks, Natick, MA) to measure action potential duration at 90% (APD_{90}), depolarization and repolarization times.

Statistical analysis

All values are presented as mean ± SEM, unless otherwise noted, with $p < 0.05$ considered statistically significant. Mean values are expressed as a percentage of baseline during control

media perfusion, before the addition of BPA. The lowest dose showing a statistically different effect ($p < 0.05$) was determined according to one-way or two-way ANOVA, followed by paired *t*-tests (Bokkers and Slob 2005). All results were computed from a minimum $n \geq 3$ independent experiments/animals for each BPA dose examined (see Table 1).

Additionally, one-way ANOVA was performed to ensure that significant differences in cardiac conduction were not present between control animals during control media perfusion, including maximum pacing frequency and conduction velocity ($p > 0.05$, data not shown).

Results

Exposure to BPA prolongs atrioventricular conduction delay

AV conduction delay was measured throughout sinus rhythm, during both control and BPA exposure. AV conduction delay remained stable during control perfusion, but lengthened significantly after BPA exposure, beginning at 10 μM (Figure 1B and C). For example, AV conduction increased by 7.5% for 10 μM BPA and 26% for 25 μM BPA exposure. Exposure to 100 μM BPA resulted in sustained and complete AV block (see Supplemental Material, Figure S1). Therefore at 100 μM BPA, conduction delays were computed immediately before the onset of AV block, which usually happened within minutes. AV conduction delays were confirmed by measuring the PR segment time in the ECG, which lengthened after 15 min of BPA exposure at all concentrations. For example, PR segment time increased by 8% for 0.1 μM BPA and 14% for 10 μM BPA (Figure 1D).

Heart block after exposure to high BPA concentrations

Exposure to 100 μM BPA resulted in 3rd degree AV block. This was detected by dropped ventricular beats, where atrium impulses failed to propagate to the ventricles (see Supplemental Material, Figure S1). Ventricular activation then resumed and was driven by an accessory pacemaker; changes in QRS morphology and lengthening were also detected in ECG recordings.

Exposure to BPA slows ventricular CV

Two-way ANOVA indicated that both pacing frequency and BPA concentration caused significant reductions in CV from control ($p < 0.0001$). The interaction of BPA and pacing frequency was also significant ($p < 0.001$), indicating that BPA altered the CV restitution properties of the tissue. At lower BPA concentrations (0.1–50 μM), significantly reduced CVs were observed at high pacing rates (Figure 2A). For example, CV for 0.1 μM BPA was 94% of control while pacing at 11 Hz. CV for 10 μM BPA was 94% of control at 7 Hz, which dropped to 86% while pacing at 11 Hz. No significant reduction in CV was measured between pacing frequencies during control perfusion (Figure 2B). In contrast, BPA exposure significantly reduced CVs, even at pacing frequencies near the intrinsic heart rate (Figure 2B). Each stimulus pulse for pacing rates from 5–15 Hz initiated a beat (1:1 capture) during control and 0.1–10 μM BPA perfusion. However, higher BPA concentrations (25–100 μM) reduced the maximum frequency of 1:1 capture (Figure 2C, left), indicating an increased refractory period. In these instances, CV was calculated using the last captured signal from a train of successfully propagated impulses (Figure 2C, right). Importantly, we did not detect significance in maximum pacing frequency or conduction velocity measurements *between animals* during control perfusion (ANOVA, $p > 0.05$, data not shown).

Optical mapping data confirmed that paced beats propagated as elliptical wavefronts that emanated from the pacing electrode, even at high BPA concentrations. An example of this is shown in Figure 2D, which illustrates reduction in CV after only 1 min exposure to 100 μM BPA. When the lengths of individual CV vectors across the epicardial field of view were averaged, it revealed a reduction of ventricular CV by 21% across the epicardial surface.

BPA exposure prolongs ventricular APD

Exposure to increasing BPA concentrations resulted in slowed CV in a rate-dependent manner, which suggested alterations in the kinetics of membrane depolarization and/or repolarization. Time intervals of depolarization and repolarization were measured using optical action potentials (Figure 3A). We found that APD_{90} was significantly prolonged (1–100 μM BPA), in a concentration-dependent manner (Figure 3B). For example, APD_{90} increased by 7% for 1 μM BPA and 44% for 25 μM BPA. Results from 100 μM BPA revealed a larger relative change in repolarization time (98% after 3rd degree AV block, Figure 3C) than depolarization time (44% after 3rd degree AV block, Figure 3D).

Discussion

Although the toxic effects of BPA on reproduction and development have been reported, a link between BPA exposure and cardiovascular disease was made only recently (Lang et al. 2008; Lind and Lind 2011; Melzer et al. 2010; Melzer et al. 2012; Shankar et al. 2012). Only a handful of studies have examined BPA's adverse cardiac effects and none have described the adverse effects of BPA on electrical conduction within intact hearts. Studies using isolated atrial preparations showed that exposure to high concentrations of BPA decreased heart rate via a nitric oxide-dependent signaling mechanism (Pant et al. 2011). Another study used isolated ventricular

myocytes to show that BPA exposure (.001 - 1 nM), when coupled with 17β-estradiol treatment, modified calcium handling (Yan et al. 2011).

Our studies were designed to show the dose-response relationship of BPA on whole-heart conduction abnormalities in female rats. We observed changes in cardiac conduction beginning at 0.1 μM BPA. This concentration is within the range of previously reported human urinary concentrations (0.024–8.5 μM, Table 1), and is within the upper limit of measured human serum levels (0.001–0.3 μM) in high-risk populations (Calafat et al. 2009; Kaddar et al. 2009; Lee et al. 2008; Padmanabhan et al. 2008; Schonfelder et al. 2002; Vandenberg et al. 2010; Wang et al. 2012). Such concentrations may be present in individuals chronically exposed to high levels of BPA (i.e., industrial workers) and those with reduced metabolic capacities (i.e., prenatal, neonatal individuals). Although BPA is not considered a persistent compound, there is controversy regarding whether or not BPA is immediately cleared from the body (Teeguarden et al. 2012; Vom Saal et al. 2012). Importantly, we observed changes in cardiac conduction following acute (≤ 15min) BPA exposure, a time frame that is significantly less than BPA's reported half life (Shin et al. 2004; Volkel et al. 2002).

We observed significant, although modest changes in cardiac conduction beginning at 0.1 μM BPA. Notably, small changes in ion channel expression and/or electrical conduction can lead to pathological outcomes (Amin et al. 2010, Grant 2009). At low micromolar concentrations (10 μM) of BPA we observed a concentration-dependent slowing of AV conduction that began within 15 min of exposure (Table 1). AV conduction delay was confirmed by measuring PR segment time, with delays beginning at 0.1 μM BPA. We also found that BPA exposure resulted in prolonged APD_{90} and slowed ventricular CV beginning at 1 μM and 0.1 μM, respectively. CV

is rate-dependent and is reduced at high pacing frequencies, a phenomenon known as CV restitution that is primarily established by the recovery kinetics of sodium channels (Kleber and Rudy 2004; Qu et al. 2004). We observed CV slowing at high pacing rates in control measurements as well as a significantly greater slowing of CV after BPA exposure. High pacing frequencies mimic increased heart rates, which could result from increased work/exercise or stress. When heart rates are elevated, individuals with increased BPA exposure could be susceptible to adverse cardiac effects, including electrical conduction that is slower than normal. In our studies we also observed that the maximum heart rate that could be achieved was significantly reduced after BPA exposure. For example, at 25 μM BPA maximum pacing frequency was reduced from 15Hz to 12.8Hz. This result indicates an increased refractory period, which could be caused by prolonged sodium channel inactivation resulting from reduced potassium current and longer action potential duration (Jalife et al. 2009).

BPA's cardiac effects were pronounced at high concentrations. 100 μM BPA exposure resulted in complete AV block and QRS interval widening, which is consistent with slowed ventricular conduction that may be attributed to reduced gap junction conductance (Gillum et al. 2009), reduced sodium channel activity, and activations that originate outside the specialized conduction system. We also detected a lengthening in APD_{90}, with a larger relative change in repolarization time than depolarization time. This indicates that BPA-induced tissue refractoriness could be the dominant mechanism of conduction failure at high concentrations and pacing rates. We recognize that cardiac exposure to 100 μM BPA exceeds a clinically relevant exposure concentration; however, reporting the observed effects may hint to BPA's underlying

mechanisms on the conduction system that can be examined further and also allow for direct comparison with previously reported mechanical effects (Pant et al. 2011).

The significance of these results is that alterations in electrical conduction is a mechanism of reentrant arrhythmias (Roden 1996), which can cause tachycardia and fibrillation in both the atria and ventricles. The effects of BPA exposure that we observed, even at low BPA concentrations, could be symptomatic at high hearts rates, particularly in elderly patients whose hearts tend to be larger and more fibrotic (Biernacka and Frangogiannis 2011). In addition, action potential prolongation is a common mechanism of conduction block, particularly at high heart rates ("rate-dependent block"), and is known to be a trigger for reentrant arrhythmias (Rosenbaum et al. 1973). Reduced conduction velocity may also cause reentrant arrhythmias, particularly in patients with dilated hearts (Akar et al. 2004). These adverse effects are important even though we did not observe such arrhythmias in our studies, which is most likely the result of the small size of the rat heart and it's intrinsically low susceptibility to arrhythmias.

Our studies compliment previous studies of the effect of BPA on mechanical function. One result of those studies was that BPA-induced mechanical dysfunction is gender-specific due to BPA's estrogenic properties (Belcher et al. 2012; Yan et al. 2011). As in these previous studies, each animal served as its own control in our studies to minimize any potential effects of estrous cyclicity. This reduced the influence of the estrous cycle on measurements of cardiac conduction, as evidenced by a lack of statistical significance in measurements between animals during control perfusion (ANOVA, $p > 0.05$, data not shown). As a result, our paired-animal controlled studies in excised hearts provide new data that reveal slowing of cardiac electrical conduction that is caused by BPA.

Such impaired electrical conduction may potentially be attributed to BPA's interaction with ion channels and/or estrogen receptors (Figure 4, and Supplemental Material, Table S1). BPA can bind directly to and block the Nav1.5 sodium channel (O'Reilly et al. 2012), which is responsible for phase 0 depolarization in ventricular myocytes (Figure 4, mechanism 1). Inhibition of the fast sodium current by BPA would certainly reduce ventricular CV. BPA can also activate Maxi-K channels in coronary smooth muscle (2; Asano et al. 2010). A similar interaction with sarcolemma potassium channels could hyperpolarize cardiomyocytes and decrease cardiac excitability. Modifications in either sodium or potassium channel current could also explain the increased tissue refractoriness we observed at high pacing frequencies.

Since BPA is classified as a xenoestrogen, it is plausible that the impaired cardiac conduction we observed was mediated by its interaction with estrogen receptors (ER) and the resultant downstream pathways. Animals that undergo ovariectomy have shortened PR intervals and shorter ventricular refractory periods (Saba et al. 2002). These observations can be explained by the effects of ER agonists on multiple ion currents. ER agonists can inhibit voltage-gated sodium current (3; Wang et al. 2013) and decrease potassium current, which can prolong APD and repolarization time (4; Berger et al. 1997; Kurokawa et al. 2008; Nakajima et al. 1999; Tanabe et al. 1999). ER agonists can also decrease L-type calcium current (5; Jiang et al. 1992; Meyer et al. 1998; Nakajima et al. 1999), which can prolong ventricular APD (Berger et al. 1997; Tanabe et al. 1999). Since L-type calcium current is the primary depolarizing current in sinoatrial and AV nodal cells, a reduction in calcium current can lead to AV block (Hancox and Mitcheson 1997; Kawai et al. 1981). Similar to other ER agonists (Jiang et al. 1992; Liew et al. 2004), BPA has also been shown to decrease cardiac contractility, possibly through its interaction with ERs

and/or activation of the nitric oxide/cGMP pathway (6; Belcher et al. 2012; Pant et al. 2011). An increase in nitric oxide levels can also attenuate L-type calcium current (Han et al. 1997), which could be an important mechanism for dropped beats and AV block at high BPA concentrations.

Conclusion

Our results show that acute BPA exposure alters electrical conduction in excised hearts from female rats. This could be the result of blocking the Nav1.5 sodium channel, reduced gap junction conductance, reduced calcium channel opening due to a nitric oxide/cGMP pathway, and/or inhibition of potassium channels via BPA's estrogenic properties. Due to the complex interactions between these pathways (Figure 4), additional studies are needed to fully identify the mechanisms responsible for BPA's effects, and to determine whether these mechanisms differ by exposure levels and treatment time.

One limitation of the current study is that the effects of BPA on cardiac conduction were examined following acute *ex vivo* exposure. Whether such conditions are relevant to chronic *in vivo* exposure is not yet known, and requires additional studies. These additional experiments are important because BPA may motivate conduction abnormalities in individuals with pre-existing heart conditions, such as AV conduction dysfunction, disease of the cardiac electrical conduction system, or fibrosis of the atrial and/or the ventricles. Other high-risk populations, such as industrial workers, prenatal and neonatal patients with reduced metabolic capacity, and elderly patients with substantial cardiac fibrosis may also be affected. Overall, our findings emphasize the importance of examining BPA's effect on heart electrophysiology and determining whether chronic *in vivo* exposure can cause/exacerbate conduction abnormalities in patients with pre-existing heart conditions and other high-risk populations.

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Table 1. Summary of statistical significance for each measured electrophysiological parameter.

Measurement	**[0.1 μM] BPA *n* = 3**	**[1.0 μM] BPA *n* = 4**	**[10 μM] BPA *n* = 4**	**[25 μM] BPA *n* = 7**	**[50 μM] BPA *n* = 7**	**[100 μM] BPA *n* = 7**	**Potential primary mechanisms of result**
Sinus rate slowing	N	N	N	N	Y	Y	Reduced Ca^{+2} current in SA node cells
AV delay	N	N	Y	Y	Y	Y	Reduced Ca^{+2} current in AV node cells
Prolonged PR segment	Y	Y	Y	Y	Y	Y	Impaired conduction in atria, AV node, and bundle branches
Reduced ventricular CV	Y	Y	Y	Y	Y	Y	Reduced Na^{+2} current and gap junction conductance
Prolonged APD	N	Y	Y	Y	Y	Y	Reduced K^{+} and Ca^{+2} currents during repolarization
Reduced max paced freq	N	N	N	Y	Y	Y	Increased ventricular refractoriness due to long APD

Significant changes ($p<0.05$) in parameter differences from control/untreated are denoted with a "Y". Insignificant changes are denoted with an "N". Potential primary mechanisms for the effect of BPA on measured parameter differences are listed in the last column. As a reference, BPA dose ranges correspond to clinical levels in blood (0.1 – 1 μM), in urine (0.1 – 10 μM) and concentrations typically used in toxicological studies (0.1 – 100 μM). The number of independent experiments/animals examined is shown for each dose.

Figure Legends

Figure 1. BPA exposure prolongs AV conduction. **A.** Heart preparation and electrode placement. LA = left atrium, RA = right atrium, RA Record = right atrium recording electrode, V Record = ventricle recording electrode **B.** AV conduction delay following control, 10 μM and 100 μM BPA exposures. **C.** Left: Time course showing AV delay following BPA exposure. Right: AV delay measured after 15 min treatment (0.1–50 μM). D**.** Left: PR segment elongation following exposure to 10 μM BPA. Right: PR segment prolongation measured after 15 min treatment. Measurements for 100 μM BPA were prior to the onset of AV block (indicated by circle). ($n \geq 3$, $^{*}p < 0.05$*).* Dose response determined by one-way ANOVA; lowest dose with significance was determined by paired t-tests.

Figure 2. BPA reduces ventricular CV. **A.** Reduced CVs at high pacing frequencies (7–11 Hz; $n \geq 3$). 0.1 μM is significantly different at 9 and 11 Hz ($^{*}p < 0.05$), but not 7 Hz ($p = 0.054$). All data points (1-100 μM) are significantly different ($^{**}p < 0.05$). **B.** CV slowing for 10 and 100 μM BPA with increasing pacing frequency ($n \geq 3$, $^{*}p < 0.05$*)*. **C.** Left: Exposure to higher BPA concentrations reduced the maximum rate of ventricular activation ($n \geq 3$, $^{*}p < 0.05$). Right: CV was calculated using the last captured signal (denoted by ‡). Pacing spikes are indicated by the ↓. The large arrow indicates loss of capture. **D.** Wavefront propagation across the ventricular epicardium (9 Hz). Local CVs are shown as arrows. **Left:** Control perfusion. **Right:** 1 min exposure to 100 μM BPA. Dose response determined by 1- or 2-way ANOVA; lowest dose with significance was determined by paired t-tests.

Figure 3. BPA prolongs ventricular APD_{90}. **A.** Optical action potentials from ventricular tissue (8 Hz). AUF = arbitrary units of fluorescence**. B.** APD_{90} was prolonged following BPA exposure ($n \geq 3$, $*p < 0.05$), likely due to longer repolarization (**C**) and depolarization time (**D)** ($n \geq 3$, $*p < 0.05$). Dose response determined by one-way ANOVA; lowest dose with significance was determined by paired t-tests.

Figure 4. Possible mechanisms underlying BPA's impairment of cardiac conduction. BPA binding can (**1)** block voltage-gated sodium channels, or **(2)** activate maxi-K+ channels (located in the mitochondria of cardiomyocytes, or in sarcolemma of cardiac neurons and endothelial cells). (**3)** ER agonists can inhibit sodium current via activation of PKC-PKA pathway. (**4)** ER agonists can inhibit potassium current. (**5)** ER agonists can inhibit L-type calcium current via NO/cGMP/PKG pathway, which acts as an antagonist to cAMP activation. This effect is concentration dependent, as NO can activate calcium channels at basal concentrations. (**6)** BPA can reduce cardiac contractility, an effect that is also dependent upon NO concentration. (see Supplemental Material, Table S1).

Figure 1

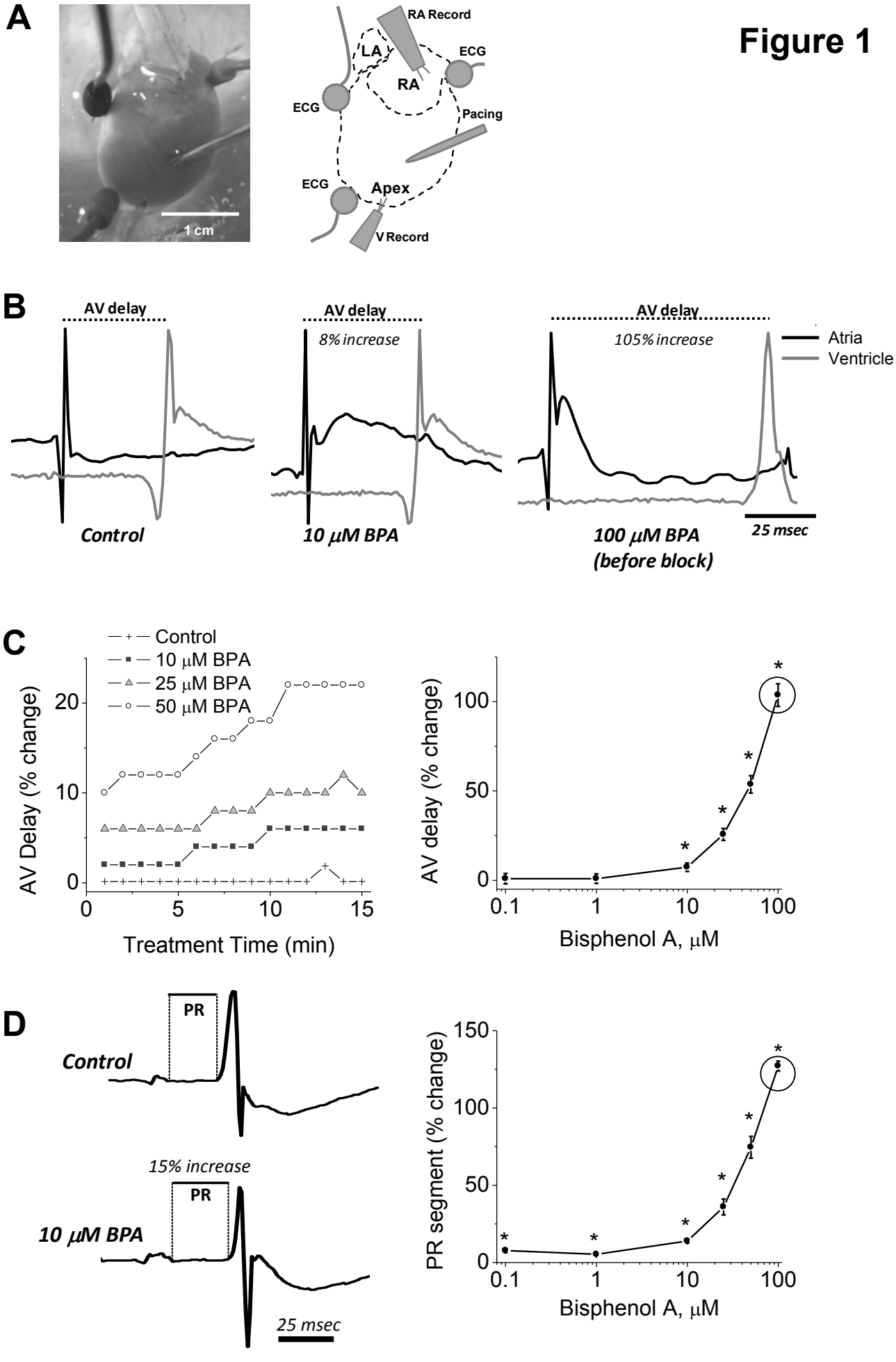
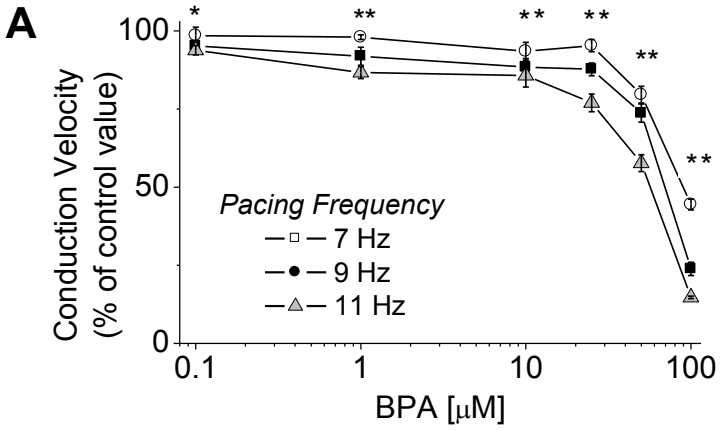
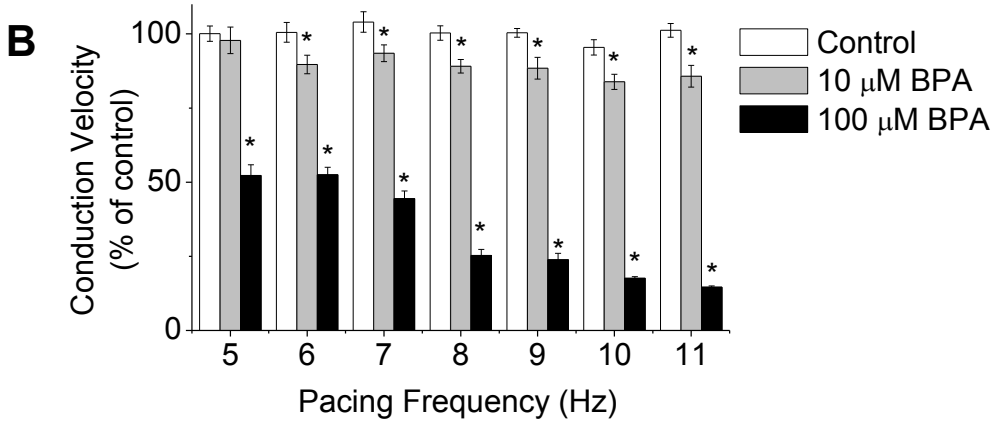

A
1 cm
RA Record
LA
ECG
RA
ECG
Pacing
ECG
Apex
V Record
B
AV delay
AV delay
AV delay
8% increase
105% increase
Atria
Ventricle
Control
10 μM BPA
100 μM BPA
(before block)
25 msec
C
Control
10 μM BPA
25 μM BPA
50 μM BPA
AV Delay (% change)
Treatment Time (min)
AV delay (% change)
Bisphenol A, μM
D
PR
Control
15% increase
PR
10 μM BPA
25 msec
PR segment (% change)
Bisphenol A, μM

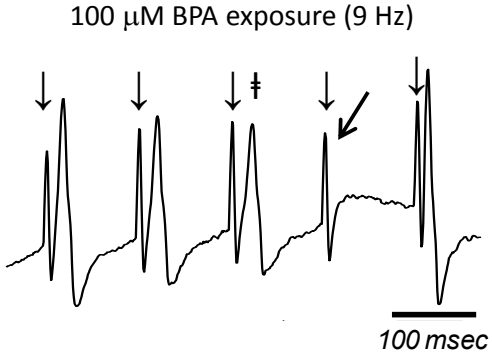
Figure 2





C

BPA, μM	Max pacing frequency (Mean ± SD)
0.1 μM	15.0 ± 0
1 μM	15.0 ± 0
10 μM	15.0 ± 0
25 μM*	12.8 ± 1.3
50 μM*	11.8 ± 1.3
100 μM*	7.5 ± 0.6



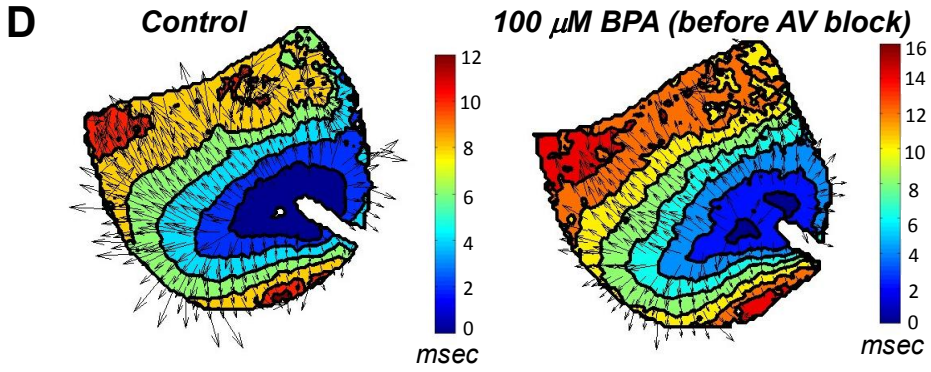


Figure 3

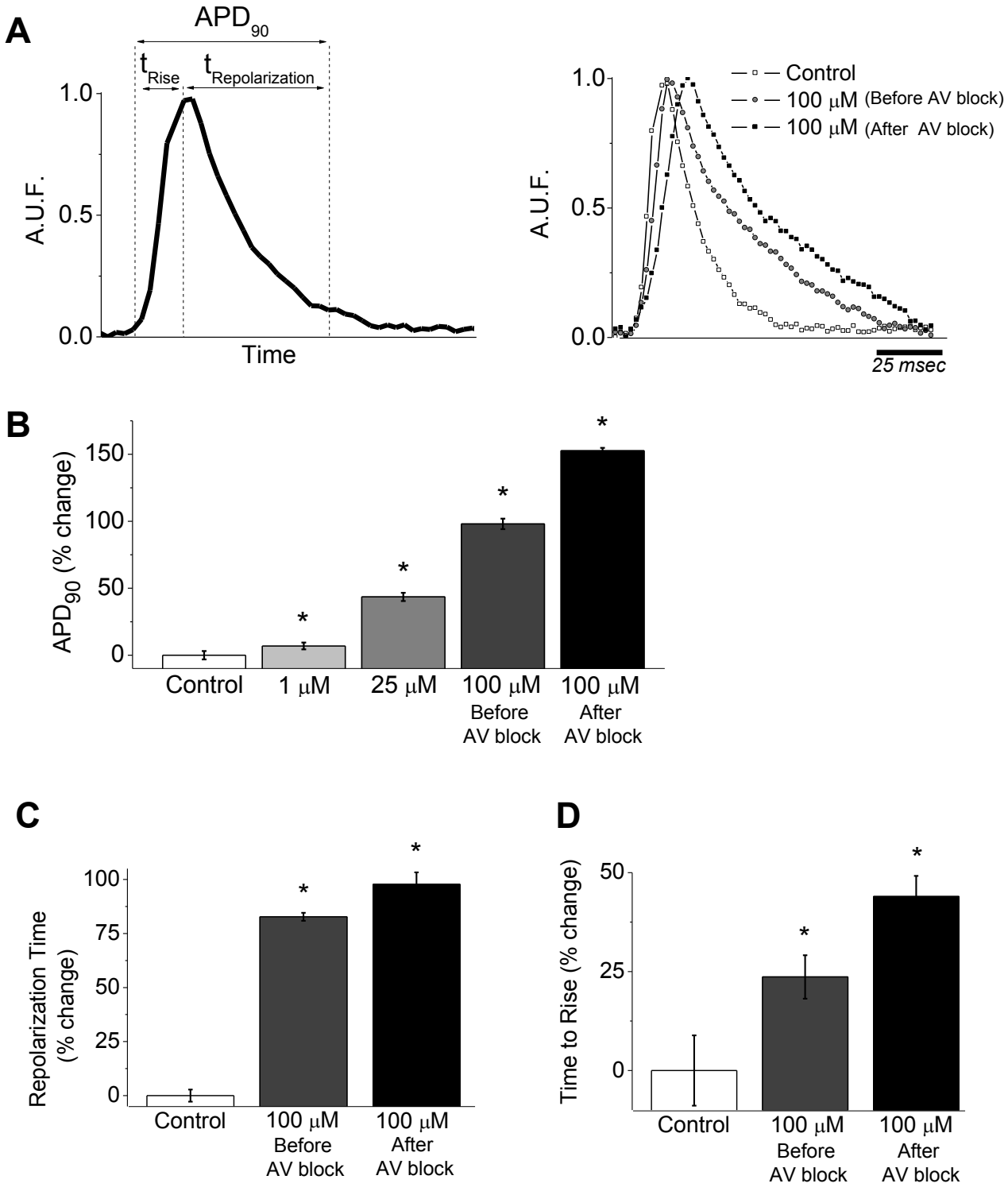
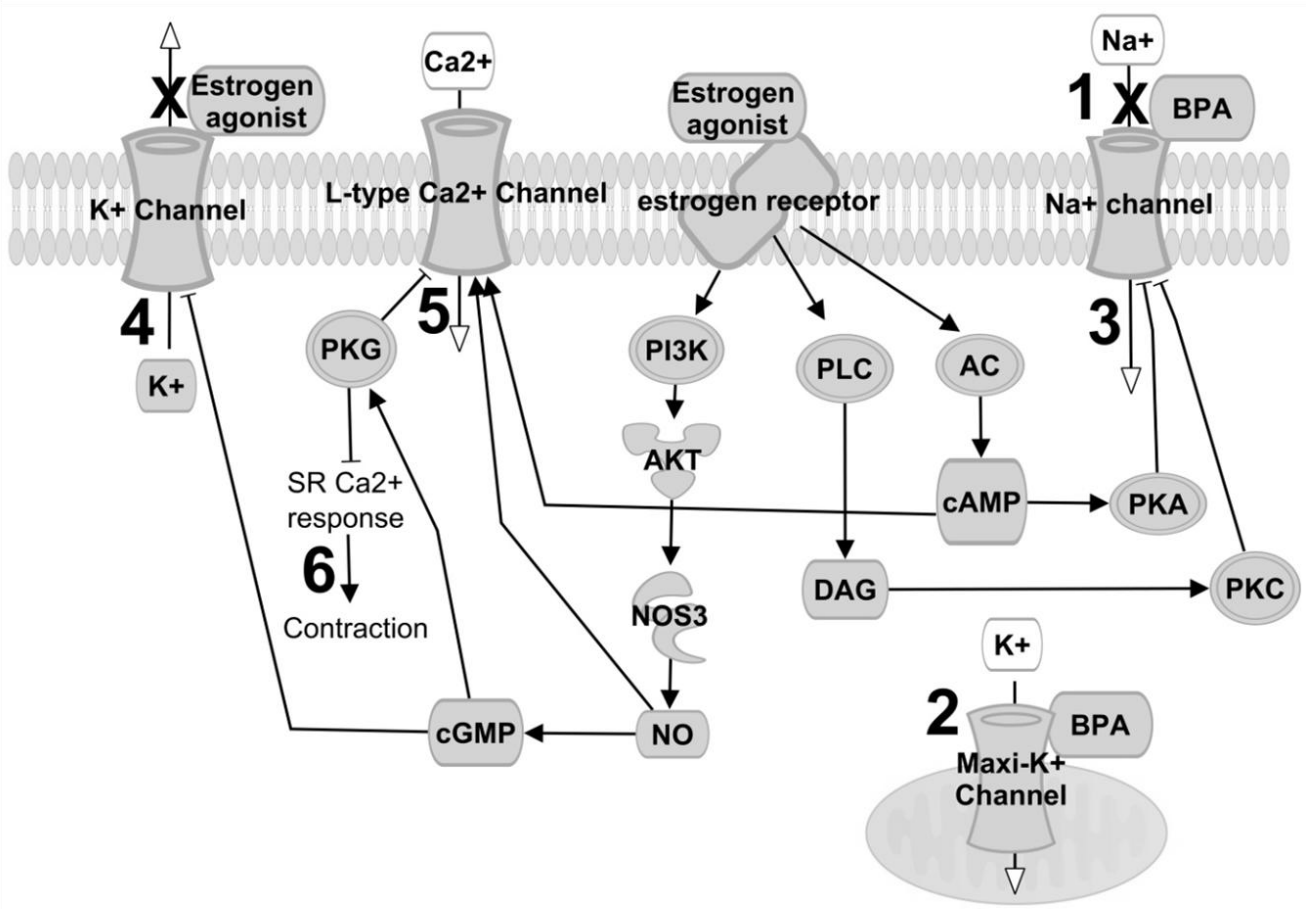

A
APD90
tRise
tRepolarization
1.0
0.5
0.0
A.U.F.
Time
Control
100 μM (Before AV block)
100 μM (After AV block)
25 msec
B
APD90 (% change)
150
100
50
0
Control
1 μM
25 μM
100 μM Before AV block
100 μM After AV block
C
Repolarization Time (% change)
100
75
50
25
0
Control
100 μM Before AV block
100 μM After AV block
D
Time to Rise (% change)
50
25
0
Control
100 μM Before AV block
100 μM After AV block

Figure 4



AC = adenylate cyclase
AKT = protein kinase B
cAMP = cyclic adenosine monophosphate
cGMP = cyclic guanosine monophosphate
DAG= diacylglycerol
PI3K= phosphoinositide 3-kinase
NO = nitric oxide
NOS3 = nitric oxide synthase 3
PKA = protein kinase A
PKC = protein kinase C
PKG = protein kinase G
PLC = phospholipase C