Sex differences and the effects of ovariectomy on the β-adrenergic contractile response

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McIntosh VJ, Chandrasekera PC, Lasley RD. Sex differences and the effects of ovariectomy on the β-adrenergic contractile response. Am J Physiol Heart Circ Physiol 301: H1127–H1134, 2011. First published June 17, 2011; doi:10.1152/ajpheart.00711.2010.—The presence of sex differences in myocardial β-adrenergic responsiveness is controversial, and limited studies have addressed the mechanism underlying these differences. Studies were performed using isolated perfused hearts from male, intact female and ovariectomized female mice to investigate sex differences and the effects of ovarian hormone withdrawal on β-adrenergic receptor function. Female hearts exhibited blunted contractile responses to the β-adrenergic receptor agonist isoproterenol (ISO) compared with males but not ovariectomized females. There were no sex differences in β1-adrenergic receptor gene or protein expression. To investigate the role of adenylyl cyclase, phosphodiesterase, and the cAMP-signaling cascade in generating sex differences in the β-adrenergic contractile response, dose-response studies were performed in isolated perfused male and female hearts using forskolin, 3-isobutyl-1-methylxanthine (IBMX), and 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (CPT-cAMP). Males showed a modestly enhanced contractile response to forskolin at 300 nM and 5 μM compared with females, but there were no sex differences in the response to IBMX or CPT-cAMP. The role of the A1 adenosine receptor (A1AR) in antagonizing the β-adrenergic contractile response was investigated using both the A1AR agonist 2-chloro-N6-cyclopentyl-adenosine and A1AR knockout (KO) mice. Intact females showed an enhanced A1AR antianti-adrenergic effect compared with males and ovariectomized females. The β-adrenergic contractile response was potentiated in both male and female A1ARKO hearts, with sex differences no longer present above 1 nM ISO. The β-adrenergic contractile response is greater in male hearts than females, and minor differences in the action of adenylyl cyclase or the A1AR may contribute to these sex differences.

Sex differences exist in the normal cardiovascular system (25, 27, 30, 33), and a role for ovarian hormones in generating sex differences in the cardiovascular system has been demonstrated using ovariectomy models (6, 19, 21). Additionally, sex differences in susceptibility to heart failure, arrhythmias, and other pathological conditions have been reported (10). Despite these reported disparities, the majority of cardiovascular research is still performed only in males.

G protein-coupled receptors (GPCRs) are important modulators of cardiac function, and are consequently key pharmacological targets, but there is limited information on sex differences in GPCR function. β-Adrenergic receptors are GPCRs with inotropic, chronotropic, and vascular effects in the heart. Few studies have investigated sex differences in cardiac β-adrenergic receptor function, and the results of these studies have been inconsistent. Controversy exists as to whether sex differences are present in β-adrenergic receptor function (5, 18, 23, 28), as well as expression (2, 4, 7, 28). However, none of these functional studies were performed in intact myocardium. Additionally, multiple studies have demonstrated the role of estrogen in modulating cardiac β-adrenergic receptor subtype expression in females (4, 12, 26).

The purpose of this study was to investigate sex differences in the β-adrenergic contractile response in the isolated perfused mouse heart. Both β-adrenergic receptor expression and direct manipulation of the activity of multiple components of its downstream signaling pathway (adenyl cyclase, PDE, and cAMP-activated pathways) were analyzed to address a mechanism for the observed sex differences. Additionally, the role of the A1AR in modulating the β-adrenergic contractile response was explored.

MATERIALS AND METHODS

Materials

Forskolin, 3-isobutyl-1-methylxanthine (IBMX), 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (CPT-cAMP), and 2-chloro-N6-cyclopentyl-adenosine (CCPA) (Sigma-Aldrich, St. Louis, MO) were prepared as 10 mM stocks in DMSO and diluted as necessary. Isoproterenol (ISO) (Sigma-Aldrich) was prepared as a 10 mM stock solution in phosphate-buffered saline with 0.1% sodium metabisulfite.

Adenosine is an autacoid whose levels increase under stressful conditions such as β-adrenergic receptor stimulation, in which metabolic demand is increased (17, 22, 31). Adenosine binds to four adenosine receptors (A1AR, A2aAR, A2bAR, and A3AR), all of which are expressed in heart. Activation of cardiomyocyte A1AR has antiadrenergic actions, attenuating the positive inotropic effect of β-adrenergic stimulation (1). Release of endogenous adenosine during β-adrenergic receptor stimulation also has antiadrenergic effects via the A1AR (8, 9).

The purpose of this study was to investigate sex differences and the effect of ovariectomy on the β-adrenergic contractile response in the isolated perfused mouse heart. Both β-adrenergic receptor expression and direct manipulation of the activity of multiple components of its downstream signaling pathway (adenyl cyclase, PDE, and cAMP-activated pathways) were analyzed to address a mechanism for the observed sex differences. Additionally, the role of the A1AR in modulating the β-adrenergic contractile response was explored.
**Animals**

The animals in this study were maintained and used in accordance with guidelines in the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication, No 85–23, Revised 1996) and evaluated and approved according to the guidelines of the Institutional Animal Care and Use Committee of the Wayne State University School of Medicine. Ten- to twelve-week-old wild-type (WT) C57BL/6 male and female mice were purchased from Jackson Laboratories (Bar Harbor, ME). Additionally, females that were ovariectomized (OvX) at 5 wk of age were purchased from Jackson Laboratories and allowed 5–7 wk for estrogen withdrawal to occur before study. Estrogen withdrawal was confirmed by comparing the uterine weight-to-body weight ratio in intact females and OvX females. Global constitutive A1AR homozygous knockout male and female mice were obtained from A1AR heterozygous knockout breeders (C57BL/6 background) provided by Jürgen Schnerrmann (National Institute of Diabetes and Digestive and Kidney Diseases), and geno-type was confirmed by tail-snip PCR.

**Isolated Heart Preparation**

*General protocol.* Hearts were excised from anesthetized (pentobarbital sodium, 60 mg/kg) and heparinized (500 units) mice, placed in ice-cold saline, and mounted on a perfusion apparatus. Hearts were perfused at constant perfusion pressure (70 mmHg) with standard Krebs-Henseleit buffer (KHB). Agonists were added directly to the perfusion buffer for all experiments. Temperature was maintained at 37°C with constant-temperature reservoirs, and by partially submerging the heart in a 37°C water-jacketed chamber filled with KHB. Hearts were paced at 420 beats/min via pacing wires inserted in the right ventricle, and a fluid-filled balloon was inserted in the left ventricle (LV) across the mitral valve and connected to a pressure transducer, permitting continuous measurement of LV pressure. The fluid-filled balloon was inflated to record a LV diastolic pressure of 5–8 mmHg. Ventricular function (LV pressure, +dP/dt, and −dP/dt), heart rate, coronary flow, and coronary perfusion pressure were continuously monitored and recorded throughout the experiments. All data were collected and analyzed using PowerLab data acquisition systems and LabChart Software for Windows (AD Instruments, Colorado Springs, CO).

*β-Adrenergic response/A1AR modulation of the β-adrenergic response protocols.* Hearts were allowed to equilibrate for ~20 min before initiation of the experimental protocols. ISO dose-response studies (1, 2, and 10 nM) were performed in paced hearts, to monitor rate-independent changes in cardiac function as well as changes in coronary flow. Hearts were either subjected to ISO infusion (control) or A1AR agonist pretreatment followed by ISO infusion. For the anti-adrenergic series of experiments, hearts were infused with 200 nM CCPA (an A1AR agonist) for 5 min before and during ISO exposure. Steady-state responses to ISO (1, 2, and 10 nM) were assessed after 2 min infusion of each dose.

Forskolin, IBMX, and CPT-cAMP dose-response protocols. Hearts were allowed to equilibrate for ~20 min before initiation of the experimental protocols. Hearts were administered forskolin, which directly activates adenylyl cyclase (100, 300, 500, 750, 1,000, and 5,000 nM); IBMX, a nonselective PDE inhibitor (0.5, 1, 10, 25, 50, and 100 μM); or CPT-cAMP, a nonhydrolyzable form of cAMP (5, 10, 25, and 50 μM), and steady-state responses in cardiac function and coronary flow were assessed after 5 min infusion of each dose.

*RNA isolation and real-time quantitative PCR.* Hearts were rapidly excised and placed in ice-cold saline, extraneous tissue (including atria) was removed, and the ventricular tissue was flash-frozen in liquid nitrogen and stored at −80°C until RNA isolation. Total RNA was isolated from ventricular tissue using Trizol reagent (Invitrogen, Carlsbad, CA). RNA was digested with DNase I and purified using the RNeasy Mini Kit (QIAGEN, Valencia, CA). RNA quantity and quality was validated by spectrophotometry and agarose gel electrophoresis. All reagents necessary for reverse transcription and real-time PCR were purchased from Applied Biosystems (Carlsbad, CA). Reverse transcription was performed on 1 μg of total RNA using the High-Capacity cDNA reverse transcription kit. Gene expression was assayed with TaqMan Universal PCR Master Mix and FAM-labeled TaqMan inventoried gene expression assays according to the manufacturer’s instructions. Real-time PCR was conducted using the StepOnePlus Real-Time PCR System. Data were analyzed by the comparative 2−ΔΔCt method, and expression of the gene of interest was calculated as a percentage of GAPDH expression.

**Membrane preparation and Western blotting.** Hearts were rapidly excised and placed in ice-cold saline, extraneous tissue (including atria) was removed, and the ventricular tissue was flash-frozen in liquid nitrogen. Samples were separated into 100,000-g cytosol and membrane fractions. Protein concentration was assayed using the Bradford method.

The 100,000-g membrane proteins were separated using 10% SDS-PAGE. Following transfer to nitrocellulose, membranes were blocked in TBST containing 5% milk. Membranes were incubated in 5% milk for 2 h at room temperature with the β1-adrenergic receptor primary antibody (1:100; Santa Cruz). After being washed, the membranes were incubated in 5% milk for 1 h at room temperature with secondary antibody conjugated to horseradish peroxidase at a dilution of 1:5,000. GAPDH was used as a 100,000-g membrane loading control. Optical density of bands was quantified using UN-SCAN-IT gel version 6.1.

**Data and Statistical Analysis**

Data were analyzed with GraphPad Prism software and presented as means ± SE. Data were analyzed using t-tests and one-way or two-way ANOVAs where appropriate. Post hoc analysis using the Newman-Keuls method was performed when necessary. Statistically significant differences in the values were taken at P < 0.05.

**RESULTS**

**Body and Tissue Weights**

WT intact females weighed less than age-matched WT males, but there were no sex differences in ventricular weight-to-body weight ratios (Table 1). While body weight and ventricular weight-to-body weight ratios were similar in OvX females compared with intact females, the uterine weight-to-body weight ratio was significantly lower in OvX females, confirming ovarian hormone withdrawal.

**β-Adrenergic Receptor Contractile Response**

There were no differences in the baseline contractile parameters between WT males and intact females (Table 2). Males had a significantly lower coronary flow than both intact and OvX females, with no differences between intact and OvX females.

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<th>Table 1. Body weight, ventricular weight-to-body weight ratio, and uterine weight-to-body weight ratio</th>
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<td><strong>Body Wt. g</strong></td>
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Data are means ± SE; n, no. of hearts. OvX, ovariectomized. *P < 0.05, male vs. intact female. †P < 0.0001, intact female vs. OvX female.
females. OvX females had a significantly lower LV systolic pressure (LVSP), +dP/dt, and −dP/dt than intact females.

The β-adrenergic contractile response to ISO (1, 2, and 10 nM) is presented in Fig. 1. Although LVSP increased upon ISO stimulation, no changes were seen in LV diastolic pressure. Males demonstrated a significantly greater contractile response to β-adrenergic receptor stimulation than intact females at both 1 and 2 nM ISO in terms of LVSP, +dP/dt, and −dP/dt. At 1 nM ISO, the percent increases in LVSP for males and intact females were 64 ± 7 and 39 ± 5%; for +dP/dt, 101 ± 15 and 54 ± 8%; and, for −dP/dt, 122 ± 14 and 67 ± 10%, respectively. There were no sex differences in the contractile responses at 10 nM ISO. Additionally, there were no differences in the contractile responses to β-adrenergic receptor stimulation between intact females and OvX females at any dose of ISO. Males showed a stronger dilatory response to ISO than did intact females and OvX females (percent increases from baseline 39 ± 5, 25 ± 3, and 23 ± 4%, respectively, at 2 nM ISO), although the flow responses were more variable and only showed statistical significance at 2 nM ISO (data not shown).

Ventricular Expression of β-Adrenergic Receptors

Real-time quantitative PCR was performed to investigate β-adrenergic receptor gene expression in ventricular tissue in males, intact females, and OvX females. β1-Adrenergic receptor gene expression did not differ between males and females (in percent of GAPDH expression, 0.16 ± 0.01 vs. 0.19 ± 0.01%), but β1-adrenergic receptor gene expression was significantly lower in OvX females (0.13 ± 0.01%) compared with intact females (Fig. 2A). There were no differences in β2-adrenergic receptor gene expression among the groups. As shown in Fig. 2, B and C, there were no differences in β1-adrenergic receptor protein expression relative to GAPDH between the three groups as determined using Western blotting.

Role of Adenylyl Cyclase, PDEs, and the cAMP-Signaling Cascade in the β-Adrenergic Contractile Response

To determine whether sex-dependent differences in β-adrenergic responsiveness were the result of differences in the activity of adenylyl cyclase, forskolin dose-response studies were performed. As shown in Fig. 3, at both the low dose of 300 nM and the high dose of 5 μM, males showed a greater contractile response (in terms of LVSP and +dP/dt) than females. However, this sex difference was not seen in −dP/dt.

The cAMP that is generated by the action of β-AR stimulation is degraded by PDEs. The nonspecific PDE inhibitor IBMX was administered to determine if there are sex differences in the basal action of these proteins. As shown in Fig. 4, males showed an enhanced response to IBMX at only 1 μM in terms of LVSP (2.4 ± 0.3% in males vs. 0.2 ± 0.7% in females), with no differences seen at any dose in +dP/dt and −dP/dt.

As shown in Fig. 5, there were no differences in the LVSP response to CPT-cAMP. Males showed an enhanced response only at 10 μM CPT-cAMP in terms of +dP/dt (56.8 ± 1.9% in males vs. 37.1 ± 6.2% in females), with no differences in −dP/dt.

Role of the A1AR in the β-Adrenergic Response

Treatment with 200 nM CCPA did not significantly change hemodynamic parameters in any group (data not shown). As shown in Fig. 6A, both males and intact females exhibited a considerable A1AR anti-adrenergic effect (compared with control contractile responses seen in Fig. 1). However, intact females demonstrated an enhanced A1AR anti-adrenergic effect compared with males. For example, CCPA pretreatment reduced the mean contractile response to 2 nM ISO by ∼60% in males and 90% in females (LVSP, +dP/dt, and −dP/dt). The potentiation of the A1AR anti-adrenergic effect in females was lost after ovariectomy (40% reduction at 2 nM ISO), and OvX females did not exhibit an A1AR anti-adrenergic effect at 10 nM ISO.

Body weight was lower in the A1AR KO male compared with WT (24 ± 1 vs. 28 ± 1 g), whereas it was higher in A1ARKO females compared with WT (23 ± 1 vs. 20 ± 1 g). Additionally, although there were no differences in the ventricular weight-to-body weight ratio in A1AR KO vs. WT males (4.8 ± 0.2 vs. 4.6 ± 0.1 mg/g), the ratio was significantly higher in A1ARKO females compared with WT females (5.6 ± 0.7 vs. 4.6 ± 0.1 mg/g).

As shown in Fig. 6B, deletion of the A1AR resulted in potentiation of the β-adrenergic contractile response in both males and females (compared with Fig. 1). In both male and female A1AR KO hearts, the LVSP response at 1 nM ISO was enhanced by 92% vs. their WT counterparts. Sex differences are still present in A1AR KO hearts in terms of LVSP and +dP/dt at 1 nM ISO. However, no sex differences were present in +dP/dt or −dP/dt at 2 nM ISO in A1ARKO hearts. Additionally, coronary flow increases were greater in males than females at all three doses of ISO.

DISCUSSION

Our results indicate that there are sex differences in ventricular β-adrenergic receptor responsiveness. Female hearts exhibited a blunted response to the β-adrenergic receptor agonist ISO compared with males, but ovariectomy did not reverse this. These differences do not appear to be the result of expression of the β-adrenergic receptor, the basal activity of PDEs, or downstream signaling activated by cAMP, but may be due, in part, to modest sex differences in expression and/or
activity of adenylyl cyclase and the $\alpha_1$AR. These findings suggest that sex differences exist in cardiac $\beta$-adrenergic receptor responsiveness, as well as in the $\alpha_1$AR anti-adrenergic effect, which will likely have important physiological consequences.

$\beta$-Adrenergic Contractile Response

Our results indicate that sex differences exist in $\beta$-adrenergic contractile responsiveness. Males exhibited enhanced contractile responses to ISO compared with intact females. Females that were ovariectomized did not display an increased $\beta$-adrenergic contractile responsiveness. Additionally, males demonstrated greater vasodilation upon $\beta$-adrenergic receptor stimulation than intact females, possibly because of the greater increase in oxygen demand that accompanies greater contractile responses.

There is debate in the literature regarding sex differences in cardiac $\beta$-adrenergic receptor responsiveness. The results of studies in rat atrial and ventricular myocytes agree with our findings that males show an enhanced $\beta$-adrenergic contractile response compared with females (6, 23, 28). In contrast, studies in isolated rat trabeculas (18) and rat papillary muscle (5) indicated no sex differences in $\beta$-adrenergic contractile responses. These disparities may be the result of differences in experimental models, as well as the doses of ISO used. As shown in Fig. 1, our studies indicated that sex differences were only apparent at 1 and 2 nM ISO. At the high dose (10 nM), these differences were no longer apparent. Our study is the first to show that sex differences exist in the $\beta$-adrenergic contrac-

Fig. 1. Hemodynamic responses to isoproterenol (ISO) in male ($n = 9$), intact female ($n = 7$), and ovariectomized female ($n = 8$) mouse hearts [left ventricular systolic pressure (LVSP, A), $+dP/dt$ (B), and $-dP/dt$ (C)]. Data are means $\pm$ SE, analyzed using one-way ANOVA for each dose of ISO. $P < 0.05$, intact female vs. male for corresponding dose of ISO (*), and ovariectomized female vs. male for corresponding dose of ISO (†).

Fig. 2. Expression of $\beta$-adrenergic receptor (AR) in ventricular tissue from wild-type male, intact female, and ovariectomized (OVX) female mice. A: relative gene expression as a percent of GAPDH ($n = 5$ for each group). Data are means $\pm$ SE. $P < 0.05$, intact female vs. ovariectomized female (†). B: Western blot for $\beta_1$-adrenergic receptor and GAPDH in ventricular membranes ($n = 3$ for each group). C: $\beta_1$-adrenergic receptor protein expression relative to GAPDH protein expression. Data are means $\pm$ SE.
tile response at low doses of ISO. These observations were made in the intact myocardium at a physiological heart rate, a model not used in previously cited studies. Our results also indicate that ovariectomy does not alter the β-adrenergic contractile response in murine myocardium. This corroborates the findings of Chu and colleagues (4), who found that ovariectomy did not change the contractile response to ISO over a wide range of doses in isolated rat papillary muscles, as well as with those of Patterson and colleagues (21) using rabbit papillary muscles. In contrast, Wu et al. (32) reported that ovariectomy increases basal contractility and β-adrenergic contractile responses in rat ventricular myocytes.

One possible mechanism for the differences in β-adrenergic responsiveness that we observed in the present study could be sex-dependent differences in β-adrenergic receptor expression. However, we observed no sex differences in levels of either β₁ or β₂-adrenergic receptor gene expression in the ventricle. Additionally, no differences were seen in protein expression of the β₁-adrenergic receptor, the primary form responsible for the contractile response to ISO. There are no other studies examining sex-dependent differences in β-adrenergic receptor expression in mouse myocardium.
myocardium. Our findings are consistent with a report that β-adrenergic receptor protein expression in the rat ventricle does not differ in males and females, as determined by both immunoblotting (5) and radioligand binding (7). However, this contrasts with other reports that males have greater β-adrenergic receptor density in rat left ventricular membranes (2, 28). Our results suggest that the sex differences in response to β-adrenergic receptor stimulation are not because of differences in β-adrenergic receptor expression.

Our results also indicate that ovariectomy did result in a slight reduction of ventricular β₁-adrenergic receptor gene expression compared with intact females, but no change in protein expression occurred. Multiple studies indicate that β₁-adrenergic receptor protein expression in rat ventricular membranes is increased after ovariectomy (13, 26, 32). Chu and colleagues (4) showed that β₁-adrenergic receptor protein expression increased following ovariectomy. However, this effect was not associated with an increase in the contractile response to ISO. It appears that the slight decrease in cardiac β₁-adrenergic receptor gene expression that we observed in OvX mice did not alter β₁-adrenergic receptor protein expression and has no significant functional effects in terms of the β-adrenergic contractile response.

Role of Adenyl Cyclase, PDEs, and the cAMP-Signaling Cascade in the β-Adrenergic Response

Because no sex differences were seen in β-adrenergic receptor protein expression, the function of proteins downstream of the receptor are likely to mediate the observed sex differences in the β-adrenergic contractile response. This study addressed the action of several components of the β-adrenergic receptor signaling cascade in both sexes and is the first to do so in the isolated perfused heart. Other investigators have examined potential sex differences in myocardial adenyl cyclase activity, using β-adrenergic receptor stimulation (7, 23, 28) to assay cAMP accumulation. However, this approach may not be ideal, based on aforementioned reports of sex differences in β-AR expression or function. Administration of forskolin to the isolated heart circumvents this issue, and specifically addresses the action of adenyl cyclase. Our results indicate a slightly enhanced contractile response in males compared with females at two doses of forskolin, a low dose of 300 nM and high dose of 5 μM. These data suggest that sex differences may exist in adenyl cyclase expression and/or activity, resulting in differential contractile responses to forskolin, particularly at the high dose administered. These differences were modest compared with the sex differences in the contractile response to ISO and suggest that the role for adenyl cyclase in generating this difference is minor.

Another potential mechanism for the sex differences in the myocardial β-adrenergic receptor responsiveness could be differences in the PDEs, which hydrolyze cAMP. In dose-responsive studies with the nonselective PDE inhibitor IBMX, there were essentially no contractile differences between male and female hearts. There have been no other studies addressing potential sex-dependent differences in myocardial PDEs, although Wang and colleagues (29) noted that there were sex differences in the vascular endothelial cell expression of PDE1A and PDE3B genes, and males showed no functional response to PDE3B inhibition, whereas females did. Another report noted that sex hormones modulate PDE5 gene expression and the vascular response to PDE5 inhibition (24). Our results suggest that there are no sex differences in PDE activity in the unstimulated heart.

To address if there are any sex differences in the contractile response downstream of cAMP generation in the isolated heart, a nonhydrolyzable form of cAMP was administered. Only +dP/dt at 10 μM CPT-cAMP showed a sex difference in the contractile response, with no differences seen in LVSP and −dP/dt. This suggests that, at equivalent concentrations of cAMP, males and females show similar contractile responses. These findings suggest that component(s) acting upstream of the cAMP/protein kinase A phosphorylation cascade are likely responsible for the contractile differences between males and females in the β-adrenergic response.
Role of the A₁AR in the β-Adrenergic Response

The anti-adrenergic actions of adenosine are well established. Adenosine reduces β-adrenergic receptor-mediated increases in myocyte contractility via the A₁AR. The results of the present study suggest that intact females display significantly greater A₁AR anti-adrenergic effects than males. This effect was observed in all contractile parameters at all doses of ISO (excluding +dP/dt and −dP/dt at 1 nM ISO). This is the first report to suggest that sex differences exist in the A₁AR anti-adrenergic effect. Although β-adrenergic responsiveness did not change following ovariectomy, the A₁AR anti-adrenergic effect did appear to be modulated at least in part by ovarian hormones, since the enhanced anti-adrenergic effect seen in females was lost following ovariectomy. In fact, ovariectomy completely blocked the A₁AR anti-adrenergic effect at 10 nM ISO.

The observed sex differences in the A₁AR anti-adrenergic effects could play a role in the reduced β-adrenergic responsiveness observed in female hearts. There is evidence that endogenous adenosine, which increases in concentration during stimulation of the β-adrenergic receptor, acts through the A₁AR to exert anti-adrenergic effects on contractility (8, 9). This serves as a negative feedback mechanism to prevent extreme responses to catecholamine stimulation. Our study is the first to report the potentiation of β-adrenergic positive inotropic responses in A₁ARKO mice compared with their WT counterparts. As shown in Fig. 6B (compared with Fig. 1), the contractile response to ISO was potentiated in both A₁ARKO males and females compared with their WT counterparts. Whereas sex differences were present in the contractile responses at 1 nM ISO, this was lost at higher concentrations. This suggests that endogenous adenosine, acting through the A₁AR, may play a part in generating sex differences in the β-adrenergic contractile response.

In conclusion, this is the first study to thoroughly investigate the cardiac β-adrenergic receptor response both at the level of receptor and downstream of the receptor in both males and females. Our results demonstrate that sex differences exist in myocardial β-adrenergic receptor function (as well as adenosine A₁ receptor function). Greater adenylyl cyclase activity or expression, as well as reduced activation of the A₁AR by endogenous adenosine, may play minor roles in contributing to the enhanced contractile response to β-adrenergic receptor stimulation in males. There are likely implications of adenosine and β-adrenergic receptor sex differences under pathological conditions such as cardiovascular disease. Adenosine receptors play a central role in cardioprotection (11), and the role of the β-adrenergic receptor in cardiovascular disease has been demonstrated (14). Interestingly, reports by Lujan et al. (15, 16) suggest that sex steroid hormones modulate the effectiveness in β-adrenergic receptor blockade in promoting coronary ischemic tolerance. Our results suggest that there are likely additional mechanism(s) beyond those we have investigated that are involved in generating sex differences in the β-adrenergic contractile response. Further studies should be performed to examine potential differences in the expression and activity of components of the β-adrenergic receptor signaling pathway such as G proteins, adenylyl cyclase, and PDEs.

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DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


