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Estrogen deficiency decreases ischemic tolerance in the aged rat heart: roles of PKCδ, PKCe, Akt, and GSK3β

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Hunter JC, Kostyak JC, Novotny JL, Simpson AM, Korzick DH. Estrogen deficiency decreases ischemic tolerance in the aged rat heart: roles of PKCδ, PKCe, Akt, and GSK3β. Am J Physiol Regul Integr Comp Physiol 292: R800–R809, 2007. First published September 28, 2006; doi:10.1152/ajpregu.00374.2006.—The mechanisms underlying the age-dependent reversal of female cardioprotection are poorly understood and complicated by findings that estrogen replacement is ineffective at reducing cardiovascular mortality in postmenopausal women. Although several protective signals have been identified in young animals, including PKC and Akt, how these signals are affected by age, estrogen deficiency, and ischemia-reperfusion (I/R) remains unknown. To determine the independent and combined effects of age and estrogen deficiency on I/R injury and downstream PKC-Akt signaling, adult and aged female F344 rats (n = 12/age) with ovaries intact or ovariectomy (Ovx) were subjected to I/R using Langendorff perfusion (31-min global-ischemia). Changes in cytosolic (s), nuclear (n), mitochondrial (m) PKC (δ, ε) levels, and changes in total Akt and mGSK-3β phosphorylation after I/R were assessed by Western blot analysis. Senescence increased infarct size 50% in ovari-intact females (P < 0.05), whereas no differences in LV functional recovery or estradiol levels were observed. Ovx reduced functional recovery to a greater extent in aged compared with adult rats (P < 0.05). In aged (vs. adult), levels of m- and nPKC(δ, ε) were markedly decreased, whereas mGSK3β levels were increased (P < 0.05). Ovx led to greater levels of sPKC(δ, ε) independent of age (P < 0.05). I/R reduced p-Akt(Ser473) levels by 57% and increased mGSK-3β accumulation 1.77-fold (P < 0.05) in aged, ovari-intact females. These data suggest, for the first time, that estrogen alone cannot protect the aged female myocardium from I/R damage and that age- and estrogen-dependent alterations in PKC, Akt, and GSK-3β signaling may contribute to the loss of ischemic tolerance. senescence; ischemia-reperfusion; female

THE SENGESCENT MYOCARDIUM HARBOURS several alterations, which negatively affect its reserve capacity to combat mechanical or ischemic stresses, even in the absence of overt cardiovascular pathology (28). That women surpass men in cardiovascular disease prevalence at approximately the same age as menopause in adults suggests that decreased plasma estradiol contributes to the age-dependent decrease in ischemic tolerance in females (1). Although animal models using surgical menopause clearly support cardioprotection from estrogen replace-
these proteins are affected by I/R remains unknown (19). Although previous studies have demonstrated estrogen-dependent cardioprotection, an important limitation of these studies is that surgically induced menopause in adult animals was used to mimic the long-term effects of aging on the hormonal milieu. As such, the combined effects of senescence and estrogen deficiency on PKC- and Akt-mediated protection from I/R remains uncertain. Accordingly, the purpose of the present study was to assess the independent and combined effects of age and estrogen deficiency on recovery from I/R and on activation of PKCe, PKCθ, Akt, and GSK3β following I/R.

METHODS

Animal care. Adult (5 mo) and aged (23 mo) female (n = 12/age) Fischer-344 rats were obtained from Harlan Sprague Dawley (Indianapolis, IN). Rats were exposed to a 12:12-h light-dark cycle and received food and water ad libitum. All animal handling and utilization were in accordance with the Penn State University Animal Care and Utilization Committee.

Surgical ovariectomy. Adult (4 mo) and aged (22 mo) female F344 rats (n = 4/group) underwent surgical ovariectomy (Ovx), according to standard procedures. Briefly, each ovary and part of the oviduct was removed, and animals were allowed to recover for 5 wk before experimental intervention. Successful surgery was confirmed by assessing circulating levels of 17β-estradiol.

I/R protocol. Animals were anesthetized with pentobarbital sodium (40 mg/kg body wt ip), and hearts were rapidly excised by midline thoracotomy and rinsed in cold (4°C) saline. After isolation and rinsing, hearts were secured to a Langendorff apparatus and perfused at 85 mmHg, according to well-established procedures in our laboratory (25, 27). During a 15-min equilibration period, pacing at 260 beats/min was established, and balloon volume was adjusted to 5–6 ml. After equilibration, hearts in the I/R group were subjected to 31-min global, normothermic ischemia, as we described previously (27). Pacing was reinitiated 1 min after restoring flow, and hearts were reperfused for 30 min. In a subset of hearts (n = 6 adult, n = 8 aged), a greater than fourfold increase in creatine kinase release (Stan Bio) was observed in coronary effluent, assuring that significant injury was induced by our experimental protocol (not shown). Following reperfusion left ventricles (LV) were isolated, weighed, halved and frozen in liquid nitrogen. LV from control hearts were harvested similarly immediately after the equilibration period. All LV sections were stored at −80°C until tissue preparation.

Trifluorometrazolium chloride staining. To assess infarct size, a subset of hearts (n = 4 adult and aged female) underwent 47 min normothermic ischemia followed by 2 h reperfusion and the area of infarction was assessed exactly as described (27). Briefly, after reperfusion, LV were isolated and frozen at −20°C for 30 min. LV were sliced transversely and stained with 1% trifluorometrazolium chloride (TTC) in phosphate buffer for 30 min at 37°C. Digital images of infarcted LV were analyzed using National Institutes of Health (NIH) ImageJ software.

Tissue sample preparation. Cytosolic, nuclear, and mitochondrial samples were prepared exactly as described previously (19, 27). Briefly, frozen LV samples were homogenized by glass-glass grinder in 10 vol of buffer A containing (in mM) 250 sucrose; 10 Tris·HCl, pH 7.4, 1 EDTA, pH 7–8, 1 orthovandate; 1 NaF; 0.3 PMSF; 5 μg/ml each of leupeptin and aprotinin; and 0.5 μg/ml of pepstatin A and antipain, and subjected to serial centrifugations of 1,000 g, 10,000 g, and 100,000 g. The 1,000 g pellet (nuclear fraction) and the 10,000 g pellet (mitochondrial fraction) were washed in buffer A and recentrifuged. The final pellets were resuspended in buffer B containing (in mM): 150 NaCl; 20 Tris·HCl, pH 7.4, 10 EDTA, pH 7–8, 1 orthovandate; 1 NaF; 0.3 PMSF and 0.5 μg/ml peptatin A, 5 μg/ml each of leupeptin and aprotinin, and 1% NP-40 and subjected to a 21,000 g centrifugation for 10 min. The resultant supernatants were defined as the nuclear and mitochondrial fractions. The 100,000 g supernatant was defined as the cytosolic fraction.

Total homogenates were prepared as described previously (19, 27). Briefly, LV tissue was homogenized on ice in cold lysis buffer containing (in mM) 20 Tris, pH 7.5, 2 each of EDTA and EGTA, pH 7.5–8.0, 5 NaF, 0.3 PMSF, as well as 5 μg/ml each of leupeptin and aprotinin, 0.5 μg/ml pepstatin A, 1 μM vanadate, 0.03% 2-mercaptoethanol, and 1% Triton X-100. After 30 min agitation on ice, samples were subjected to a 100,000 g centrifugation at 4°C, and the supernatant was taken as the total fraction. All protein concentrations were determined by the method of Bradford (9).

Western blot analysis. Western blot analysis was performed according to well-established procedures in our laboratory (25, 26). Briefly, equal amounts of cytosolic (PKC and cytochrome c), nuclear (PKC), mitochondrial (PKC, GSK3β), or total homogenate (Akt) were electrophoresed on SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes. Membranes were blocked and incubated with rabbit polyclonal antibodies against PKCθ (1:1,200) or PKCε (1:1,500) for 3 h at room temperature or against p-AktSer473, Akt, p-GSK3βSer9, GSK3β, cytochrome c (1:1,000) overnight at 4°C. Immunoreactive bands were detected using HRP-linked anti-rabbit IgG (1:15,000) and enhanced chemiluminescence (Amersham). Densitometry was performed using Scion Image (NIH).

To control for total GSK and Akt protein levels, membranes were initially incubated with phospho-specific antibodies [p-Akt (Ser473)], or p-GSK3β (Ser9), and then stripped by 30 min incubation (70°C) in buffer containing 62.5 mM Tris·HCl pH 6.80, 7% 2-mercaptoethanol, 2% SDS. Membranes were subsequently probed for total levels of Akt and GSK3β, as described above.

To control for minor differences in protein loading, all membranes were stained with Sypro Ruby (PKCδ, PKCε) or Ponceau S (p-Akt, p-GSK3β, Akt, GSK3β), and densitometry values were adjusted, as described previously (39). All samples were normalized to adult female control.

Statistical analyses. All data are presented as means ± SE and analyzed using the Statistical Analysis System (SAS). Baseline data were compared with a two-way ANOVA. A two-way ANOVA with repeated measures on one factor (age × reperfusion time) was used to analyze functional recovery and a two-way ANOVA (age × group) was used to analyze Western blot analysis data. The Tukey-Kramer method was employed for all post hoc comparisons. An alpha level of P ≤ 0.05 was defined as statistically significant.

RESULTS

Rat heart characteristics. Table 1 shows morphological and baseline cardiac functional data for adult and aged female rats with gonads intact or following ovariectomy. LV and body weights were significantly greater in aged animals compared with adults. As reported previously, Ovx led to a further increase in body weight in aged females (P < 0.05), and a small but significant increase in left ventricular developed pressure (LVDP) and +dP/dt in both adult and aged animals (P < 0.05) (38).

Recovery after I/R. Recovery of LVDP (A and B), +dP/dt (C and D), and −dP/dt (E and F) are displayed in Fig. 1. Although a modest reduction in LVDP recovery (23%) was observed in adult Ovx females, the effects of estrogen deficiency were more pronounced in aged animals, resulting in a significant reduction in LVDP recovery throughout reperfusion (P < 0.05) (Fig. 1B). Reductions in +dP/dt and −dP/dt were also observed early in reperfusion (63% and 69%, respectively; P < 0.05) in Ovx (compared with intact) aged females (Fig. 1, D and F). The only age-dependent reduction in postischemic
function in females with ovaries intact was noted with respect to $\frac{dP}{dt}$ ($P < 0.05$) (Fig. 1F). A similar pattern for age-related reductions in coronary flow during reperfusion following Ovx were also observed (group × reperfusion time interaction, $P < 0.001$; Fig. 2).

Figure 3 shows that Ovx had no effect on recovery of end diastolic pressure (EDP) during reperfusion in adult females (A), whereas EDP remained elevated two- to threefold in aged Ovx (vs. ovary-intact) females throughout reperfusion ($P < 0.05$; B). Of interest, increasing the time of ischemia to 47 min or increasing the perfusate-free Ca$^{2+}$ concentration to 2 mM reduced functional recovery (50% and 30%, respectively) similarly in both adult and aged gonad-intact females (data not shown). Despite the lack of age-dependent functional decre-

Table 1. Morphological and baseline functional characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Adult Female</th>
<th>Adult Female Ovx</th>
<th>Aged Female</th>
<th>Aged Female Ovx</th>
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<tr>
<td>Body weight, g</td>
<td>210.1±2.4</td>
<td>212.3±5.9</td>
<td>255.8±6.2*</td>
<td>320.8±19.8*†</td>
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<tr>
<td>LVW, mg</td>
<td>558±12</td>
<td>548±13</td>
<td>760±15*</td>
<td>837±64*</td>
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<td>LVW/body weight, mg/g</td>
<td>2.65±0.05</td>
<td>2.59±0.05</td>
<td>2.97±0.07*</td>
<td>2.60±0.07†</td>
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<tr>
<td>EDP, mmHg</td>
<td>5.57±0.05</td>
<td>5.60±0.12</td>
<td>5.52±0.07</td>
<td>5.52±0.08</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>134.1±2.1</td>
<td>143±6.1†</td>
<td>133.1±1.9</td>
<td>142.5±6.8‡</td>
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<td>$+\frac{dP}{dt}_{\text{max}},$ mmHg/s</td>
<td>3712±102</td>
<td>4239±237‡</td>
<td>3701±124</td>
<td>4194±280‡</td>
</tr>
<tr>
<td>$-\frac{dP}{dt}_{\text{max}},$ mmHg/s</td>
<td>2400±38</td>
<td>2294±61</td>
<td>2261±50</td>
<td>2319±43</td>
</tr>
<tr>
<td>Plasma estradiol, pg/ml</td>
<td>27.9±2.8</td>
<td>7.2±0.85‡</td>
<td>26.9±1.8</td>
<td>6.5±0.92‡</td>
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</tbody>
</table>

Results are presented as means ± SE. Ovx, ovariectomized; EDP, end diastolic pressure. LVDP, left ventricular developed pressure; LVW, left ventricular weight; *$P < 0.05$ effect of age within ovary status; †$P < 0.05$ effect of Ovx within age; ‡$P < 0.05$ main effect of Ovx.

Fig. 1. Functional recovery after ischemia/reperfusion (I/R). Hearts were subjected to 31-min normothermic global ischemia, and recovery of function was followed throughout 30 min of reperfusion. To quantify recovery, the following variables were measured: percent recovery of left ventricular developed pressure (LVDP; A and B), $+\frac{dP}{dt}$ (C and D), and $-\frac{dP}{dt}$ (E and F). For clarity, adult recovery (A, C, E) is presented separately from aged recovery (B, D, F). All results are presented as means ± SE. *$P < 0.05$ aged different from adults with ovaries intact. †$P < 0.05$ effect of ovariectomized (Ovx) within aged vs. adult.
ments in females with ovaries, infarct size was significantly increased by 50% ($P < 0.05$) in aged (vs. adult) females (Fig. 4). It is important to note that increased area of infarction in aged females was observed despite comparable plasma estradiol levels (Table 1, $P = \text{NS}$). Importantly, Ovx in aged animals further increased infarct size by 35% as compared with 25% increase in adult animals, which corresponded to subsequent decrements in LV functional recovery (Fig. 1B). A similar pattern was observed for cytosolic cytochrome $c$ levels (Fig. 5). Finally, the area of infarction in adult females subjected to 47 min ischemia was comparable with the infarction observed in adult males after 31 min ischemia (data not shown), consistent with the concept of female-dependent cardioprotection.

PKC subcellular distribution. Figure 6A shows that aged females had significantly higher levels of cytosolic PKC$\delta$ compared with adult females in control rats (1.4-fold), and in post-I/R ovariectomized rats (1.65-fold) ($P < 0.05$). Conversely, cytosolic PKCe was reduced in post-I/R aged (vs. adult) rats with and without ovaries (30%, $P < 0.001$) (Fig. 6B). Furthermore, Ovx significantly increased post-I/R cytosolic PKCe in both adult and aged female rats (1.5-fold, $P < 0.001$).

Figure 7A demonstrates that nuclear PKC$\delta$ levels were unchanged by I/R and Ovx in aged female rat hearts. Levels of nuclear PKC$\delta$ were higher in adult (vs. aged) intact (1.4-fold, $P < 0.005$), and Ovx (1.25-fold, $P = 0.076$) animals after I/R. In contrast, senescence was associated with lower levels of nuclear PKCe at baseline (40%) and after I/R in both intact and Ovx animals (55%; $P < 0.05$) (Fig. 7B). Additionally, in adult and aged females, levels of nuclear PKCe were significantly lower in post-I/R Ovx compared with control rats ($P < 0.05$).

I/R led to a 26% decrease in mitochondrial PKC$\delta$ levels in intact adult female rats ($P < 0.05$) but had no effect in aged rats (Fig. 8A). Furthermore, the combination of Ovx and I/R led to a significant decrease in both adult and aged mitochondrial PKC$\delta$ ($P < 0.05$). Figure 8B demonstrates that aged (intact) females had 45% less mitochondrial PKCe baseline compared with adult (intact) females ($P < 0.005$). This difference was negated after I/R by the 40% reduction in mitochondrial PKCe in adult (but not aged) intact females. Similar differences (from control) in mitochondrial PKCe levels were noted in post-I/R Ovx females.

Akt activation. In agreement with previous reports, the present study demonstrated higher levels of p-Akt (Ser473) in the total homogenate (1.7-fold) in females compared with males, independent of age (data not shown) (11). Age-dependent increased levels of p-Akt (Ser473) were noted at baseline in total (1.56-fold) and nuclear homogenates (not shown); however, this pattern was reversed after I/R in gonad-intact

Fig. 2. Relative coronary flows after I/R. Hearts were subjected to 31-min normothermic global ischemia, and coronary effluent was collected throughout 30 min of reperfusion. All data are presented as means ± SE. *$P < 0.05$ different from adult, †$P < 0.05$ effect of Ovx within aged.

Fig. 3. Recovery of end-diastolic pressure (EDP) after I/R. Hearts were subjected to 31-min normothermic global ischemia, and recovery of EDP was followed throughout 30 min of reperfusion. All data are presented as means ± SE. †$P < 0.05$ effect of Ovx within age.
females ($P < 0.05$) (Fig. 9A). Additional age-dependent differences were noted in the response to Ovx in which only adult animals demonstrated a reduction (38%) in post-I/R p-Akt levels after Ovx (compared with post-I/R intact females) ($P < 0.05$). Although total levels of Akt were lower in aged vs. adult rats at all time points studied ($P < 0.05$, Fig. 9B), correcting for total levels of Akt (Fig. 9C) did not change the pattern of Akt activation observed in Fig. 9A.

**GSK3β phosphorylation.** Similar to ovariectomy-dependent changes in post-I/R levels of p-Akt, phosphorylation of post-I/R mitochondrial GSK3β was significantly reduced by Ovx only in adult animals (Fig. 10A). It is important to note that total levels of mitochondrial GSK3β were 1.6- to 2.7-fold greater in senescent (vs. adult) females in all groups (Fig. 10B) and that in intact females, I/R significantly increased total levels of mitochondrial GSK3β only in the aged (1.76-fold, $P < 0.001$). Unexpectedly, total levels of post-I/R mitochondrial GSK3β were significantly reduced by Ovx in both adult and aged myocardium (58%, $P < 0.05$). Taken together, Fig. 10C shows that the relative inactivation of GSK3β is greatly reduced (52–67%) in aged vs. adult rats independent of ovary status ($P < 0.005$). Of interest and consistent with changes in p-Akt levels, we observed significantly greater p-GSK3β in females vs. males ($n = 4$; $P < 0.05$; data not shown).

**DISCUSSION**

Postmenopausal women are especially vulnerable to ischemic insult and ～80,000 more cardiovascular deaths occur yearly in aged women than in aged men. The age-dependent reversal of female cardioprotection may be independent of sex hormone levels, as aged women in placebo-controlled trials have not demonstrated the well-documented cardioprotective advantage of estrogen-replacement observed in animals (2, 8, 15, 18, 41). This discrepancy may be due to the use of surgical menopause in adult animal models, which may obscure important age-estrogen deficiency interactions. Accordingly, we sought to determine the individual and combined effects of age and estrogen deficiency on functional recovery from I/R and associated signaling in female rats. Key findings are as follows: 1) age was associated with greater infarct size independent of changes in plasma estradiol levels and in spite of similar functional recovery between adult and aged groups, 2) Ovx resulted in a more pronounced reduction in posts ischemic functional recovery in aged vs. adult rats, and 3) profound reductions in mitochondrial and nuclear PKCε were observed in aged rats and were associated with increased levels of active mitochondrial GSK3β independent of estrogen deficiency. These results suggest, for the first time, that estrogen alone is insufficient to protect the aged female myocardium from I/R damage.

The majority of studies investigating age-dependent decrements in ischemic tolerance have been conducted in male animals and have demonstrated reduced ischemic tolerance with age (29, 46). In contrast to studies by Willems et al. (49), who studied aging in female mice, we found that recovery of systolic function following I/R was not different between adult and aged female F344 rats with ovaries intact after either 31 or 47 min of ischemia. A notable difference between the present
The preservation of post-I/R systolic function in spite of greater infarct size in gonad-intact aged animals is intriguing for several reasons. First, although stunning may account for the preservation of postischemic function observed in adult and aged hearts, this interpretation is confounded by the more than four-fold increase in postischemic creatine kinase release (not shown) and the presence of necrotic (TTC) staining. Of interest, this apparent disconnect between function and damage is reminiscent of heart failure in women in which systolic function can be preserved despite the presence of pathological hypertrophy (12, 44). However, further studies are required to conclude whether this disconnect truly occurs following I/R in females. Second, the age-dependent increase in area of infarction in the present study was noted in the absence of significant changes in plasma estradiol with age. Although it is important to note that we did not control for day of estrus, since aged female rats were likely in a state of constant estrus (33), our results suggest that preserved levels of estrogen are not sufficient to prevent age-dependent increases in post-I/R area of infarction.

To determine the combined effects of senescence and estrogen deficiency, adult and aged females were ovariectomized 5 wk before I/R. The Ovx-induced increase in body weight in aged females resulted in an LV-to-body weight ratio comparable to aged male F344 rats (reported previously in Ref. 27). That this increase in body weight was not observed in adults...
may suggest that estradiol may be more important in maintaining LV/body wt in aged vs. adult rats. The relative decrease in LV mass (to body weight) in the aged due to Ovx may have contributed to the greater reductions in post-I/R recovery by limiting the reserve capacity of the heart. Furthermore, Ovx resulted in greater ischemic intolerance in aged (vs. adult) rats, suggesting that aged females may be more dependent on the cardioprotective effects of endogenous estrogens than adults. Although previous studies have shown I/R-dependent functional decrements with Ovx in adult females (16, 24), to our knowledge, we are the first to address recovery from I/R in senescent, Ovx females. Taken together, these findings might have clinical implications, as it is most often the aged who experience both estrogen deficiency and ischemic cardiac episodes.

Previously, we demonstrated in male rats that age-associated reductions in ischemic tolerance were ameliorated with PKCε inhibition in a GSK3β-dependent manner (27). These results, together with the opposing cardioprotective effects of PKCδ and PKCε (20), led us to investigate whether perturbations in PKCε, PKCδ, Akt, or GSK3β signaling may underlie age- and estrogen-dependent differences in I/R recovery.

Here, the observation that significant I/R-induced PKC translocation occurred in intact adult (but not intact aged) rats...
is intriguing and consistent with previous studies demonstrating age-dependent blunting of PKC translocation in response to phenylephrine or ischemic preconditioning (25, 45). These data suggest that reduced translocation of PKCs may underlie the decreased ischemic tolerance (i.e., increased size of infarction) observed with age in female rat hearts. This situation may be further exacerbated by the present findings that I/R in combination with Ovx tends to shift PKC partitioning from the nuclear and mitochondrial fractions to the cytosolic fraction. The cytosolic fraction has classically been thought of as an inactive pool of PKC. However, we and others have demonstrated active PKC in the cytosol using phospho-specific antibodies that detect Ser/Thr phosphorylation at the activation loop and hydrophobic domains of PKCδ and PKCe (26, 36, 42). Furthermore, studies have demonstrated that PKCδ may be active in the cytosol due to tyrosine phosphorylation (43). Although PKC phosphorylation was not measured in the present study, it is conceivable that the increased cytosolic partitioning of PKC due to Ovx leads to activation of novel signals downstream of phosphorylated cytosolic PKCδ and PKCe, which may exacerbate myocardial damage.

Further compromising the ability of the aged heart to respond to an ischemic stress is the observation in the current study that following I/R, aged females have lower levels of PKC (δ and ε) at the nucleus compared with adult females. Because both PKCδ and PKCe promote cardiac hypertrophy (10, 13), these findings may represent age-dependent reductions in the ability of the aged heart to induce compensatory ventricular remodeling (47). These results are consistent with several studies that have documented a reduced cardiac hypertrophic response in aged rats following pressure or volume overload (4, 21).

Like PKCδ and PKCe, Akt promotes hypertrophy and is involved in cardioprotection (23, 31). Furthermore, Akt has been shown to be activated by estrogen (11). Akt can mediate protection at the mitochondria by phosphorylating and inactivating GSK3β and Bad, both of which promote apoptosis when active (23, 34). Our present findings of age-dependent increases in total p-Akt contrast the findings of Camper-Kirby and colleagues (11), who noted a significant age-dependent decrease in nuclear p-Akt levels. This discrepancy is likely due to differences in species and/or plasma estradiol levels (no age-dependent reductions in estradiol levels were noted in the present study). Our observation that I/R reduced levels of p-Akt (Ser473) in aged, but not adult, gonad-intact females supports a protective role for Akt and suggests that the decrease in active Akt with senescence may contribute to the increased area of infarction observed in aged (vs. adult) rat hearts. Because chronic Akt activation also mediates hypertrophy (31), the age-dependent increase in basal p-Akt levels may represent an underlying mechanism of the relative hypertrophy observed with aging.

One target of Akt that has been proposed as a convergence point for cardioprotective signaling is GSK3β (23). This model is supported by the present observation of I/R- and ovarectomy-dependent changes in pGSK3β, which mirror changes in pAkt in adult female rats. In contrast, changes in p-Akt levels are disconnected from changes in p-GSK3β levels in the aged, which might suggest that Akt-dependent GSK3β phosphorylation is dysfunctional in the aged. Alternatively, GSK3β phosphorylation may be occurring independently of Akt, as proposed by Juhaszova and colleagues (22). That mitochondrial levels of total GSK3β were elevated in aged (vs. adult) intact females at baseline and were significantly increased by I/R only in the aged may contribute to the increased area of infarction observed in the aged, gonad intact females. Of interest, recent studies suggest that GSK3β can enhance ERα-mediated transcription (32). The age-dependent increase in mitochondrial GSK3β in the present study may represent a translocation away from the nucleus. If this is so, several cardioprotective proteins that are modulated by estrogen [such as nitric oxide synthase (35), ATP-dependent K+ channels (40), and heat shock proteins (48)] may show diminished expression, thus contributing to the decrease in ischemic tolerance in the aged.

In summary, our results demonstrate that senescence, independent of alterations in plasma estradiol levels, leads to reduced ischemic tolerance in the female rat myocardium and that ischemic intolerance is exacerbated by estrogen deficiency to a greater extent in the aged. Our results further suggest that alterations in post-I/R distribution and activation of PKCδ, PKCe, Akt, and GSK3β may underlie age- and estrogen deficiency-dependent reductions in both acute recovery and long-term remodeling of the female rat heart.

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REFERENCES

