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ORIGINAL PAPER

Sex Differences in Ischemia/Reperfusion Injury: The Role of Mitochondrial Permeability Transition

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Abstract

Brain and heart ischemia are among the leading causes of death and disability in both men and women, but there are significant sex differences in the incidence and severity of these diseases. Ca^{2+} dysregulation in response to ischemia/reperfusion injury (I/RI) is a well-recognized pathogenic mechanism leading to the death of affected cells. Excess intracellular Ca^{2+} causes mitochondrial matrix Ca^{2+} overload that can result in mitochondrial permeability transition (MPT), which can have severe consequences for mitochondrial function and trigger cell death. Recent findings indicate that estrogens and their related receptors are involved in the regulation of MPT, suggesting that sex differences in I/RI could be linked to estrogen-dependent modulation of mitochondrial Ca^{2+}. Here, we review the evidence supporting sex differences in I/RI and the role of estrogen and estrogen receptors in producing these differences, the involvement of mitochondrial Ca^{2+} overload in disease pathogenesis, and the estrogen-dependent modulation of MPT that may contribute to sex differences.

Keywords Ischemia · Sex · Mitochondrial permeability transition · Calcium · Estrogen · Estrogen receptor

Sex Differences in Ischemia/Reperfusion Injury

Ischemia, the blockage of blood supply to a part of the body, is a critical and prevalent public health problem. Common forms of ischemia are cerebral ischemic stroke (IS), caused by a blockage in blood flow to the brain, and myocardial ischemia (MI), caused by a blockage in the heart. Sex differences in both cerebral and MI have attracted considerable attention in recent years and are now well documented. Ischemic heart disease is the leading cause of death in both women and men [1]. Stroke, which includes both hemorrhagic and ischemic causes (although IS is far more common [2]) has dropped to the fifth leading cause of death in men, but remains the third in women [3]. There are clear age-related sex differences in both IS and MI incidence, as after age-matching it becomes apparent that women are protected from IS/MI until approximately age 75, when risk reverses and women become significantly more vulnerable [2, 4–6]. Strikingly, the overall lifetime incidence of stroke is higher in women [7, 8], and 60% of all stroke deaths occur in women [9]. Sex differences exist not only in the incidence of ischemia, but also in its severity. Regardless of age, women have more severe strokes and worse post-stroke outcomes [8, 10]. They are more likely to be disabled by stroke, have higher mortality rates [11], and are 3.5 times more likely to be placed in a nursing home post-stroke than men [3]. Similarly, after acute myocardial infarction, women are more likely to experience independence loss and poorer quality of life [12, 13]. Furthermore, some therapeutic strategies for IS, such as neuronal nitric oxide synthase (nNOS) and poly (ADP-ribose) polymerase (PARP) inhibitors, have been demonstrated pre-clinically to have better efficacy in males than females [3, 6], underscoring the importance of understanding the mechanisms underlying sex differences in IS/MI, so effective therapies can be developed for all patients.

Sex differences in IS/MI incidence and severity could partially derive from environmental factors, such as psychosocial risk factors [13] and a tendency for women to have atypical clinical presentation, resulting in delayed diagnosis

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and treatment [9]. However, there is a clear biological component, because sex differences are recapitulated in multiple animal models of ischemia/reperfusion injury (I/RI). Adult female mice have smaller brain lesions than males in a model of moderate hypoxia/ischemia [14]. Females are also preferentially protected in other widely used rodent models of cerebral ischemia [15], including the most commonly used middle cerebral artery occlusion (MCAO) model, which produces a lesion in the caudate/putamen and cortex in the hemisphere ipsilateral to occlusion. Interestingly, MCAO produces a significantly larger infarct and poorer functional motor recovery in aged female rats when compared to young females [16], experimentally recapitulating the increased vulnerability of older women to IS. Similar trends can be seen in the heart, as female rat hearts are more resistant than male hearts to damage after oxygen deprivation [1] and tend to have smaller infarcts after myocardial I/RI in the Langendorff preparation [17].

The effects of sex and age on the incidence and outcomes of IS/MI point to a role for sex hormones. Estrogens are a demonstrably important part of the sex differences, as risk for ischemia in women correlates well with lifetime fluctuations in estrogen levels. Women are protected from both IS and MI before menopause, but risk increases steeply after menopause or oophorectomy, when estrogen declines [1, 18, 19]. Additionally, women with natural menopause before age 42 have double the risk for IS than women with later menopause [20]. Estrogen replacement therapy (ERT) would seem an effective way to offset this increase in risk. Beneficial effects of ERT have been reported [18], but ERT is still controversial, because the Women's Health Initiative (WHI) ERT clinical trial was discontinued after an increase in adverse effects, including cardiovascular disease. There is evidence that ERT may be effective if administered before age 50 [19], and post hoc analysis of the WHI data demonstrates that the therapy is advantageous when given to women recently post-menopause, but not to women who had been menopausal for many years [21]. Therefore, it would be premature to completely discount ERT as an option for reducing the risk of ischemia until more research is done to outline the precise conditions affecting its efficacy.

Pre-clinical studies using animal models have directly demonstrated that estrogens are important in the early protection and age-related increase in vulnerability to I/RI in females. The estrogen estradiol (17-beta estradiol, 17βE) is neuroprotective in ovariectomized female rats undergoing transient MCAO [22]. Additionally, hypertensive female rats, prone to spontaneous strokes, are more susceptible to neuronal damage after permanent MCAO in metestrus (low circulating estradiol) than in proestrus (high estradiol), although estradiol levels did not affect ischemic brain damage in another, non-hypertensive strain of rats [23]. Consistent with the re-analysis of the WHI study, estrogens are only neuroprotective in I/RI when given soon after ovariectomy in rats, as they lose all beneficial effects when given after a 10-week period of hypoestrogenism [24]. These data fit well with the estrogen-correlated resistance to IS seen clinically in premenopausal women, but also suggests that estrogen neuroprotection is not straightforward, and more work needs to be done to elucidate its mechanisms. Estrogen plays a role also in MI, as isolated cardiac myocytes from female rats are more resistant to simulated I/RI than male myocytes, but myocytes from older or ovariectomized rats lose this protection [25]. Moreover, 17βE is protective in myocardial I/RI in both female and male rabbit hearts [18, 26], indicating that exogenous estrogen could also be protective in males. These data indicate that animal models of myocardial I/RI can mirror the age- and hormone-related effects observed in patients with MI.

In addition to estradiol, estrogen receptors (ERs) are also involved in producing the sexually dimorphic phenotypes observed in I/RI. There are two canonical ERs, ERα and ERβ, that have both genomic (transcriptional) and non-genomic actions, and a more recently discovered G-protein coupled receptor with a high binding affinity for estrogens termed GPER. Both ERα and ERβ are involved estrogen-mediated neuroprotection. The synthetic ERα -selective agonist PPT and the ERβ -selective agonist WAY 200070-3 both attenuate neuronal death in the CA1 region of the hippocampus in ovariectomized rats after transient global ischemia [27]. Furthermore, 17βE, but not the hormonally inactive 17αE, exerts protective effects in myocardial I/RI of ovariectomized rabbits, demonstrating an ER-dependent mechanism [28]. GPER activation with the agonist G1 is also protective in myocardial I/RI in males [29], and estradiol mediates protection of ischemic male hearts through GPER [30], providing further evidence that estrogens/ERs exert protection in both males and females. Taken together, data from humans and animal models provide strong evidence that estrogen is protective against IS and MI, and that the decline of estrogen after menopause puts females at a particularly high risk.

Calcium-Dependent Mechanisms of Cell Death in I/RI

Differences in the mechanisms leading to cell death could contribute to the sex differences in the clinical outcomes of I/RI. Cell death in I/RI is a complex process involving multiple pathways. A well-recognized trigger of cell death in I/RI is a pathological rise of intracellular calcium (Ca^{2+}), and evidence shows that estrogens and ERs can modulate Ca^{2+}-dependent ischemic cell death. In neurons, this type of cell death is mainly mediated by glutamate excitotoxicity, which is defined as a glutamate-mediated accumulation

of intracellular Ca^{2+} that produces cell damage [31]. 17βE prevents glutamate excitotoxicity in hippocampal and cortical neurons [32, 33] and reduces lesions following stereotactic perfusion of glutamate into the cerebral cortex of male rats [34]. DPN, an ERβ-selective agonist, is neuroprotective against excitotoxic glutamate in hippocampal neurons [35]. Interestingly, neurons from ERβ knockout animals are protected from glutamate excitotoxicity and oxidative stress [33, 36]. 17βE also prevents intracellular Ca^{2+} loading after hypoxia/reoxygenation in female cardiomyocytes [37].

During glutamate excitotoxicity Ca^{2+} enters the cytosol most notably through NMDA-type ionotropic glutamate receptors [38, 39], but also through additional routes, including other ionotropic glutamate receptors and voltage-gated Ca^{2+} channels (VGCCs), as well as release from endoplasmic reticulum stores induced by metabotropic receptors [40]. In the ischemic heart, a massive accumulation of intracellular Ca^{2+} occurs through an increase in the reverse activity of the plasma membrane Na^+/Ca^{2+} exchanger, which allows Ca^{2+} into the cell and extrudes Na^+. The reason for this reversal is a steep decline in intracellular pH due to increased glycolysis, which forces the Na^+/H^+ exchanger to pump H^+ ions out of the cell, while allowing large amounts of Na^+ in [41]. Non-Na^+ dependent pathways of Ca^{2+} influx, including VGCCs, also appear to play a role in the heart [42]. Because efflux mechanisms depend on the plasma membrane Ca^{2+}-ATPase pump and Na^+/Ca^{2+} exchanger operating in forward mode and sequestration in the ER by the sarco/endoplasmic reticulum Ca^{2+}-ATPase [40], ATP depletion during ischemia precipitates intracellular Ca^{2+} accumulation [43]. Figure 1 schematically illustrates the main mechanisms of cytosolic Ca^{2+} regulation in neurons and cardiomyocytes during I/RI.

The cytosolic accumulation of Ca^{2+} during I/RI leads to Ca^{2+} uptake by mitochondria. Mitochondria import Ca^{2+} into the matrix through the mitochondrial Ca^{2+} uniporter (MCU), which is regulated by several associated proteins [44]. Efflux of Ca^{2+} from the matrix involves both Na^+-dependent (through the mitochondrial Na^+/Ca^{2+} exchanger) and Na^+-independent (through the Ca^{2+}/H^+ exchanger) pathways. Figure 1 illustrates the key players of mitochondrial Ca^{2+} regulation. The capacity of mitochondria for Ca^{2+} import exceeds their efflux capacity, leading to mitochondrial accumulation of Ca^{2+} when cytosolic Ca^{2+} is high [45]. This Ca^{2+} accumulation is functionally important in ischemia because it plays a key role in the excitotoxic

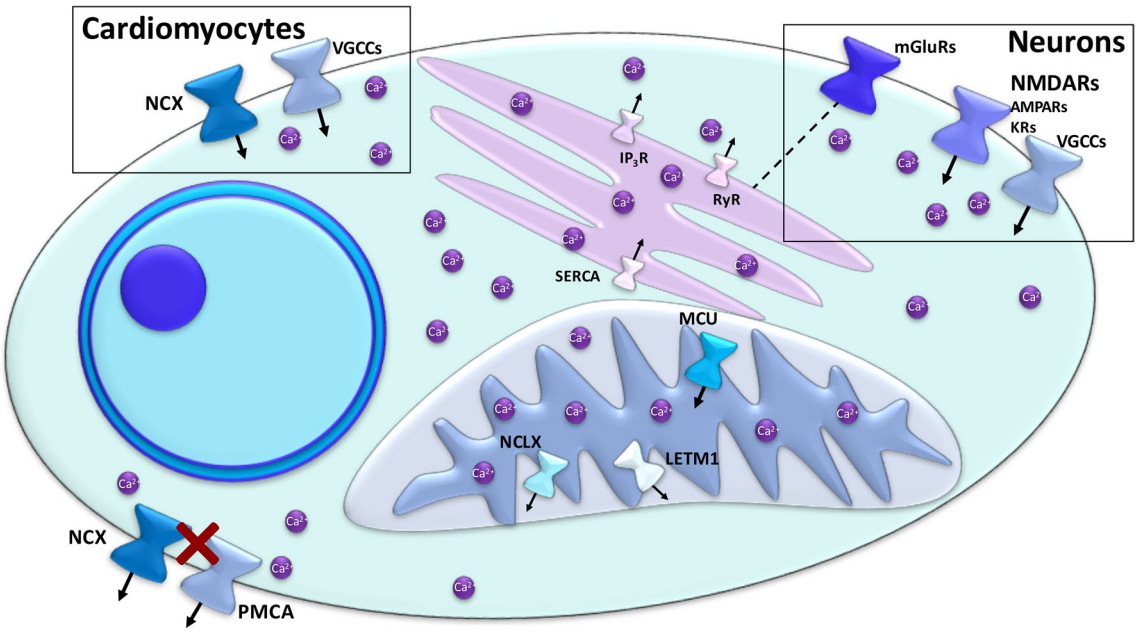

Fig. 1 Modes of cellular Ca^{2+} entry and release in I/RI. During ischemia, Ca^{2+} enters cardiac cells through the plasma membrane Na^+/Ca^{2+} exchanger (NCX) operating in reverse mode, and through voltage-gated Ca^{2+} channels (VGCCs). In neurons, cytosolic Ca^{2+} influx occurs mainly through ionotropic NMDA-type glutamate receptors (NMDARs), other ionotropic glutamate receptors (AMPARs, KRs), and VGCCs. Additionally, in both neurons and cardiomyocytes, the endoplasmic reticulum can take up Ca^{2+} through the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and release stored Ca^{2+} through inositol triphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs). In neurons, ER Ca^{2+} release can be induced by metabotropic glutamate receptors (mGluRs). Intracellular Ca^{2+} overload occurs because efflux requires the NCX operating in forward mode or energy-dependent plasma membrane Ca^{2+}-ATPases (PMCA), both of which fail in ischemia. Mitochondria are depolarized during ischemia, so Ca^{2+} import is minimal, but Ca^{2+} is imported into the matrix upon repolarization during reperfusion. Import occurs through the mitochondrial Ca^{2+} uniporter (MCU), and efflux occurs through the mitochondrial Na^+/Ca^{2+} exchanger (NCLX) and Ca^{2+}/H^+ exchanger (LETM1)

neuronal death occurring in IS [46, 47] and in the mitochondrial dysfunction that precedes cell death in myocardial I/RI [42]. Blocking mitochondrial Ca^{2+} uptake largely decreases neuronal death after excitotoxic glutamate exposure [48, 49]. Furthermore, functional, polarized mitochondria are necessary for restoring intracellular Ca^{2+} homeostasis after excitotoxic stress [50]. Taken together, these data suggest that mitochondrial accumulation of Ca^{2+} is highly relevant for the Ca^{2+}-mediated death of neuronal and cardiac cells in I/RI and could be an important contributor to sex differences in ischemia.

Mitochondrial Permeability Transition (MPT) and its Role in I/RI

High amounts of matrix Ca^{2+} cause severe damage to mitochondria, and by extension to the cell as a whole, through a well-studied but still incompletely understood mechanism termed the MPT. Mitochondria rely on a highly impermeable inner mitochondrial membrane (IMM) to generate the proton motive force essential for ATP production. Matrix Ca^{2+} overload triggers MPT, a process mediated by the opening of a non-specific pore, which causes a sudden disruption in the impermeability of the IMM (Fig. 2) [51, 52].

The MPT pore (MPTP) has a conductance of approximately 1.5–2 nS, an estimated diameter between 2 and 3 nm, and can allow the passage of solutes smaller than 1.5 kDa through the IMM [53, 54]. A major consequence of MPT is the collapse of the mitochondrial membrane potential, which results in a loss of ion homeostasis and proton motive force and therefore depletion of ATP generation capacity and subsequent energy failure [54]. Additionally, when the MPTP opens the colloidal osmotic pressure of the proteins in the matrix results in a large influx of water, causing swelling which can burst mitochondrial membranes, facilitate the release of cytochrome c, and initiate apoptosis (Fig. 2) [54, 55]. The main inducer for MPTP opening is high matrix Ca^{2+} [56], but other factors including matrix depolarization, neutral matrix pH, oxidative stress, inorganic phosphate, and

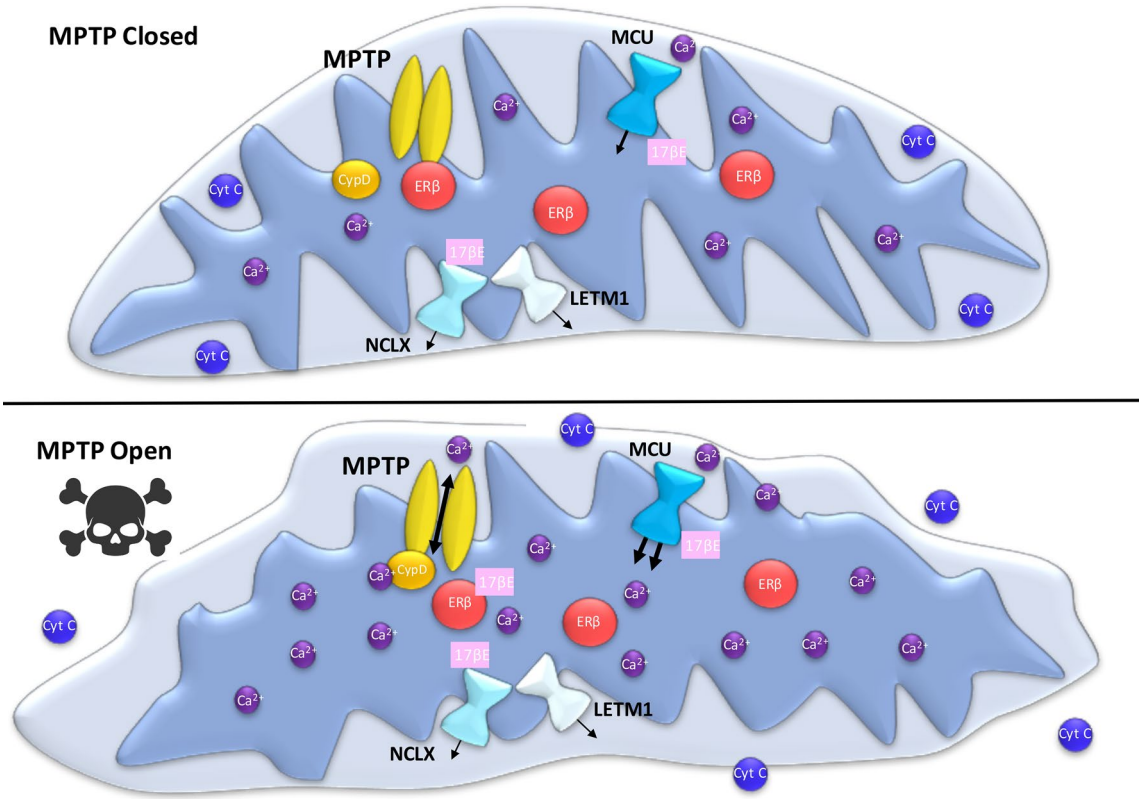

Fig. 2 17βE and ERβ regulate MPT in I/RI. Mitochondria take up cytosolic Ca^{2+} into the matrix through the MCU and release Ca^{2+} through NCLX and LETM1. 17βE can affect matrix Ca^{2+} levels by modulating both MCU and NCLX. In healthy mitochondria, matrix Ca^{2+} does not reach the threshold for triggering MPT and the MPTP remains closed. Therefore, mitochondria maintain membrane integrity, energy production, and cell viability is preserved. During I/RI, matrix Ca^{2+} overload causes opening of the MPTP, resulting in MPT and collapse of mitochondrial energy-generating capacity. MPTP opening causes matrix swelling, rupture of mitochondrial membranes, and release of pro-apoptotic factors, such as cytochrome C, resulting in cell death. 17βE and ERβ can modulate MPT under conditions of I/RI by regulating both matrix Ca^{2+} levels and the MPTP

de-energization can lower the Ca^{2+} threshold and increase MPTP opening probability [57, 58]. Factors decreasing MPTP opening probability are acidic matrix pH, adenine nucleotides, and magnesium [59]. The regulation of MPT is complex, because it is cell-type and context-specific and multiple interacting factors mediate MPTP opening. For example, matrix pH, inorganic phosphate levels, and mitochondrial energization state are highly interconnected factors that regulate MPT [54, 56, 58]. These are particularly relevant in the context of I/RI, because there are extensive and rapid changes in mitochondrial energization state and matrix pH in the different stages of ischemia and reperfusion.

The structural makeup of the MPTP remains controversial. The adenine nucleotide translocator ANT was originally believed to be a structural component of the pore [60], but genetic loss of function experiments ruled this out [61]. However, there is evidence that ANT may play a regulatory role in the Ca^{2+} threshold for pore opening, particularly in response to matrix depolarization and oxidative stress [54, 62]. The c subunit of the F1 region of the F0/F1 mammalian ATP synthase has been suggested as a pore forming component [63, 64]; however, simulations showed that the c-ring is thermodynamically unfavorable to occupation by water and therefore would be unlikely to form a pore [65]. The interface between F0/F1 ATP synthase dimers has also been suggested to form the MPTP [66, 67], but MPT can occur in cells with all three genes encoding subunit c of the ATP synthase knocked out [68] and in cells lacking either subunit b or the oligomycin-sensitivity conferring protein (OSCP) subunit of the ATP synthase [69]. Nevertheless, it is possible that multiple subunits would need to be knocked out simultaneously to fully abolish MPT.

While the proteins forming the pore itself remain unknown, CypD is a widely accepted pore regulator. CypD is a peptidyl-prolyl isomerase localized to the mitochondrial matrix [70]. CypD-deficient mitochondria are resistant to Ca^{2+}-induced MPT, identifying it as a Ca^{2+} sensor for the pore [71–73]. Cyclosporin A (CSA), a fungal-derived compound, is the most widely used inhibitor of MPT, and modulates pore opening by inhibiting CypD [74, 75]. However, CSA also inhibits all other cyclophilins, leading to powerful immunosuppressive and potentially neuromodulatory effects through its inhibition of calcineurin; so, results obtained with CSA must be interpreted with caution [70, 76]. CypD has been shown to interact with the OSCP subunit of the ATP synthase in an inorganic phosphate-stimulated and CSA-sensitive manner [77, 78]. This interaction lowers ATP generation capacity by uncoupling the ATP synthase from the electron transport chain and lowering its catalytic activity [79]. Loss of OSCP causes a CypD-independent increase in MPTP opening [80], suggesting that if the ATP synthase is a component of the pore, MPTP modulation by CypD could be dependent on its interaction with OSCP.

While MPT was traditionally thought of as an all-or-nothing event occurring exclusively in pathological situations, a large body of evidence suggests that the MPTP can also have reversible low-conductance states, which may play a physiological role. Low-conductance states (approximately 0.5 nS) with permeability to solutes under 300 Da have been measured in isolated mitochondria and in cells [75, 81–85] and in vivo in astrocytes [86]. Additionally, low conductance reversible opening of the MPTP in cardiac mitochondria is protective against Ca^{2+} overload [87]. Mitochondria have limited Ca^{2+} efflux mechanisms, which can easily become overloaded during periods of high Ca^{2+} entry into the cell. Transient MPTP opening could provide a fast and thermodynamically favorable route for mitochondrial Ca^{2+} efflux, although its role as a Ca^{2+} release channel is controversial [45, 88, 89]. MPT is also a variable and highly heterogeneous phenomenon across the mitochondrial pool, as not all mitochondria in a cell will undergo the transition simultaneously or at all [90]. Furthermore, some populations of mitochondria appear to be more vulnerable to MPT. For example, neuronal synaptic mitochondria have a lower Ca^{2+} threshold than non-synaptic mitochondria [91, 92]. This heterogeneity could be explained in part by variations in the expression of CypD, whose levels have been shown to differ based on subcellular localization, tissue, and age [77, 92, 93].

MPT has been demonstrated to play a crucial role in cell death in a variety of pathological conditions, but most notably in I/RI [94, 95]. Although reperfusion is necessary to limit cell death due to hypoxia during ischemia, it can also promote MPT. While mitochondria are depolarized during ischemia, Ca^{2+} accumulates in the cytosol. After reoxygenation mitochondria reestablish their membrane potential, allowing for massive Ca^{2+} entry into the matrix. Ca^{2+} influx depolarizes the matrix, which, together with the depletion of adenine nucleotides, a burst of reactive oxygen species, and reestablishment of matrix pH, primes mitochondria for MPT [96].

There is convincing evidence that blocking CypD-sensitive MPT is protective in models of ischemia. CSA treatment significantly decreases infarct size after transient MCAO [97] or global brain ischemia [98], and CypD knockout dramatically reduces infarct size in MCAO [71]. Inhibiting MPT at the time of reperfusion with the non-immunosuppressive CypD inhibitor NIM811 protects the heart against I/RI [99], and CypD knockout reduces infarct size by up to 40% [100, 101]. Furthermore, mitochondria isolated from CypD knockout animals are resistant to swelling induced by high Ca^{2+}, and CypD overexpression in the heart increases the probability of mitochondrial swelling, cardiac cell death, and cardiac hypertrophy [101]. Interestingly, CypD deficient hepatocytes and embryonic fibroblasts are protected from necrosis but not against several apoptotic stimuli [71, 100, 102]. Taken together, this evidence

suggests that CypD-regulated MPT is an important contributor to necrotic, but not apoptotic, cell death in I/RI. However, a role for MPT not mediated by or insensitive to CypD in apoptotic cell death cannot be ruled out.

Sex Differences in Mitochondrial Ca^{2+} Handling and Permeability Transition

The regulation of protection by estrogens and ERs that is observed in I/RI must be mediated at least in part through their maintenance of mitochondrial function, as the neuroprotective ability of estrogenic compounds correlates highly with their ability to maintain mitochondrial membrane potential after insult [103]. Some studies have addressed this issue directly. For example, it was shown that mitochondria from ERβ knockout animals repolarize and maintain ATP production more effectively than wild type controls after oxidative insult [36]. In addition, estradiol and ER ligands can directly modulate mitochondrial Ca^{2+} uptake and release dynamics. 17βE, DPN, and ERα-selective agonist PPT increase Ca^{2+} uptake into the matrix through the MCU in a receptor-independent fashion, while tamoxifen, an ER antagonist, inhibits it [104]. In rat synaptosomal mitochondria, 17βE at physiological concentrations decreases Na^{+}-dependent Ca^{2+} efflux, but at higher concentrations increases it [105]. Estradiol can modulate the ability of mitochondria to tolerate high Ca^{2+} loads, as it was shown to increase the levels of Bcl-2 protein [106]. 17βE also prevents age-related Ca^{2+} dysregulation in neurons from male rats by preserving the ability of mitochondria to accumulate Ca^{2+} [32]. Altogether, this suggests that males and pre- or post-menopausal females may possess distinct dynamics of mitochondrial calcium homeostasis, which could contribute to sex differences in I/RI.

Importantly, sex differences have been demonstrated in MPT. Female mitochondria from both brain and spinal cord have lower Ca^{2+} capacity, a measure of susceptibility to MPT, and greater Ca^{2+} induced depolarization. Genetic ablation of CypD abolishes these differences, suggesting that they are dependent on MPT [33, 107]. Additionally, treating both female and male brain mitochondria with a high concentration of 17βE decreases their Ca^{2+} capacity, and this was prevented by CypD knockout, pointing to a direct role for 17βE in modulating MPT [107]. These results demonstrate that female brain mitochondria are more prone to MPT than male brain mitochondria. Further, mitochondria isolated from female rat heart have higher Ca^{2+} capacity than male mitochondria [108], but also take up less Ca^{2+} initially, have reduced swelling in response to high matrix Ca^{2+}, and recover membrane potential more quickly after Ca^{2+}-induced depolarization [109, 110]. These results suggest that female cardiac mitochondria are less susceptible to MPT than male mitochondria, while female brain mitochondria are more susceptible, suggesting that sex-dependent regulation of MPT is tissue-specific.

In addition to estradiol, ERβ is an important mediator of the susceptibility of female brain mitochondria to MPT and could potentially modulate MPT sensitivity in other tissues as well. We have shown that knockout of ERβ decreases the sex difference in brain mitochondrial Ca^{2+} capacity, and that this effect depends on CypD. ERβ localizes in various cell compartments, including the mitochondrial matrix [111], suggesting that the mitochondrial pool of ERβ could be responsible for modulating MPT. We showed that ERβ knockout decreases the interaction between CypD and OSCP, a proposed MPTP component, while 17βE increases it, likely by acting as a ligand for ERβ and modulating its protein–protein interactions [33]. In addition, 17βE binds to OSCP directly [112]. At pharmacological concentrations 17βE promotes the "slip" rate of the ATP synthase by decreasing the efficiency of the coupling between proton flow and ADP phosphorylation [113]. Therefore, modulation of the CypD–OSCP interaction by 17βE and/or ERβ could provide the mechanism for the estrogen-dependent sex differences in MPT.

Conclusions and Future Perspectives

IS/MI are common causes of disability and death that pose a severe burden for public health worldwide, and this will only increase as the population continues to age. It is clear that sex differences exist in ischemic injury, due to the modulatory effects of estrogen and ERs. Understanding the basis for these differences would provide insight into fundamental mechanisms of cell death in I/RI that can be targeted to develop therapeutics effective in both sexes. Ca^{2+}-dependent cell death is a crucial part of the injury, in both brain and heart. One of the main mechanisms mediating this type of cell death is mitochondrial Ca^{2+} overload and MPT. Estrogen/ER-dependent sex differences in mitochondrial Ca^{2+} handling and MPT are likely linked to the sexual dimorphism seen in IS/MI. Based on studies by our group and others, we propose that sex differences in MPT are related to the modulation of the interactions between CypD and MPTP components by 17βE and ERβ. Figure 2 schematically summarizes the processes leading to mitochondrial Ca^{2+} overload and MPT in I/RI, the downstream consequences of MPT, and the proposed modulatory role of estrogens and ERβ on mitochondrial Ca^{2+} dynamics.

In the future, it will be important to better understand the extent to which estrogens and ERs modulate the sexual dimorphism in ischemia. Estrogens and ERs are undoubtedly involved, but sex differences could also result from other hormones including androgens, as well as genetic

and epigenetic dissimilarities. More work needs to be done to directly compare in both sexes the influence of estrogens and ERs on mitochondrial function, Ca^{2+} handling, and MPT, in physiological and pathological conditions. More research is also necessary to examine the mechanisms by which estrogens and ERs regulate MPTP opening. Identifying the targets of 17βE/ERβ in modulating MPT will provide more clarity about the true components of the pore, as well as new approaches to regulate MPTP opening and promote cell survival in I/RI. Additionally, it is still unclear whether the sex differences in MPT reflect variations in irreversible high conductance MPTP opening, reversible low conductance opening, or both. Reversible MPTP opening could potentially be a protective mechanism against mitochondrial Ca^{2+} overload by allowing limited ionic efflux. Whether reversible MPTP opening occurs differentially in I/RI in males and females, and if it is modulated by estrogens and ERs, remain to be elucidated. This will be important when considering targeting the MPTP as a therapeutic strategy in a sex-dependent manner. Finally, it will be important to investigate whether sex differences in MPT play a role in other pathologies besides ischemia. Many neurodegenerative diseases have significant differences in occurrence and progression in men and women, including Alzheimer's disease, amyotrophic lateral sclerosis, and multiple sclerosis, and early evidence indicates that MPT could be an important contributor to pathogenesis in these disorders [107, 114, 115]. However, whether differences in MPT and its modulation by estrogen and ERs contribute to the sex differences in these diseases is yet to be determined.

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