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Review

Sex shapes experimental ischemic brain injury

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ABSTRACT

Biologic sex and sex steroids are important factors in clinical and experimental stroke. This review evaluates key evidence that biological sex strongly alters mechanisms and outcomes from cerebral ischemia. The role of androgens in male stroke is understudied and important to pursue given that male sex is a well known risk factor for human stroke. To date, male sex steroids remain largely evaluated at the bench rather than the bedside. We review recent advances in our understanding of androgens in the context of ischemic cell death and neuroprotection. We also highlight some possible molecular mechanisms by which androgens impact ischemic outcomes.

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1. Stroke: a sexually dimorphic disease

We have only recently come to understand that stroke or "Brain attack" is a sexually dimorphic disease, i.e. the outcomes and mechanisms of this form of brain injury are strongly linked to biological sex. Male sex is a well-acknowledged risk factor for human stroke, although little is known about the mechanism(s) behind this observation. The overall incidence of stroke is higher in men vs. women in most nations, a trend that cuts across ethnic background [1]. This sexually dimorphic epidemiology is present in children [2] and persists until ages well beyond the menopausal years [3,4], suggesting that sex steroids are not the controllers of male vs. female risk for disease. For example, stroke rates in women do not equalize to those of men until beyond 75 years of age [4]. However, stroke risk does increase with age in both sexes, and there is some evidence that outcome from an ischemic event is worse in aged women than in their male counterparts (for reviews [5,6]). Therefore, knowledge of mechanisms of ischemic cell death and neuroprotection is important for both sexes. As discussed below, recent data suggest that ischemic pathobiology is strongly influenced by sex in experimental animal models, and cellular and molecular death mechanisms may not be identical in the male vs. female (for additional recent reviews [7–9]).

2. Sex and animal stroke models

Animal models have been used to evaluate side-by-side effects of cerebral ischemia, and in most reports, there are clear sex differences in outcomes [10–14]. Early evidence in female vs. male spontaneously hypertensive, genetically stroke prone rats

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Table 1
Sex-specific cell death mechanisms in ischemic brain injury.

Cell/species	Insult/mechanism tested	Sex difference	References
Dopaminergic neurons	High concentrations dopamine	Female cells have greater survival vs. male	[21]
Cortical plate neurons; ventricular zone neurons	Longevity in culture, expression of protective kinases	Female cells survive longer, respond to injury with higher kinase expression	[22]
Primary neuronal culture	Neurotoxins, including glutamate, ONOO, H_2O_2, staurosporine	Higher sensitivity in males to glutamate-ONOO injury; females are more sensitive to apopototic challenges	[23]
Cortical astrocytes	OGD with or without inflammatory stimulus (TNFα; IL1β); role of P450 aromatase	Greater survival of female cells after OGD, higher aromatase expression, higher ability to synthesize cytoprotective estradiol	[24,25]
Cultured hippocampal slice	OGD, NMDA exposure; NO toxicity	Less injury in females slices; only male slices received benefit from nNOS inhibition	[26]
Intact male vs. female mouse brain	Focal cerebral ischemia; role of nNOS, PARP	Male but not female brain is protected by genetic loss or pharmacological inhibitors of nNOS, PARP and downstream mechanisms	[14]
Intact male vs. female mouse brain	Focal cerebral ischemia; role of apoptotic mediators	PAR formation and AIF nuclear translocation occurs in both sexes, but resulting apoptotic damage only in males	[33]
Intact neonatal male vs. female mouse brain	Hypoxia–ischemia; role of PARP	Male but not female brain is protected by genetic loss of PARP	[29]
Intact neonatal male vs. female mouse brain	Moderate but not severe hypoxia–ischemia; role of AIF and caspase activation	More pronounced AIF nuclear translocation in male brain; caspase activation greater in female brain	[30]
Intact male vs. female mouse brain	Focal cerebral ischemia; role of iNOS	Male but not female brain is protected by genetic loss or pharmacological inhibition of iNOS	[35]
Intact neonatal male vs. female rat brain	Focal cerebral ischemia; role of caspase activation	Female but not male brain was protected by pharmacological broad spectrum, caspase inhibition	[31]
Young and aged male vs. female intact mouse brain	Focal cerebral ischemia; role of cytochrome *C* release and caspase activation	Earlier cytochrome *C* release in female vs. male brain; female brain but not male brain was protected by pharmacological broad spectrum, caspase inhibition	[32]
Intact male vs. female mouse brain, expressing transgenic luciferase gene, GFAP promoter	Focal cerebral ischemia; role of astrocyte activation	Early brain inflammation as measured by GFAP intensity is higher in female vs. male; unlike male, GFAP intensity does not correlate with tissue damage in female	[37]

Abbreviations—AIF: apoptosis-inducing factor; GFAP: glial fibrillary protein; H_2O_2: hydrogen peroxide; iNOS: inducible isoform nitric oxide synthase; IL1β: interleukin 1-β; nNOS: neuronal isoform nitric oxide synthase; NMDA: N-methyl-D-aspartic acid; OGD: oxygen-glucose deprivation; ONOO: peroxynitrate; PAR: poly-ADP-ribose; PARP: poly-ADP-ribose polymerase; TNFα: tumor necrosis factor α.

uncovered the male phenotype of "ischemia-sensitivity" [15]. This landmark study of 2000 animals showed that life expectancy is longer in the female rat, and the development of spontaneous stroke lesions is delayed until an advanced age [15]. Female rats and mice of various inbred and outbred strains experience smaller tissue damage for an equivalent insult from focal or global cerebral ischemia and improved functional outcomes [10,11,14,16]. Similarly, male animals sustain greater injury than age-matched females after traumatic brain injury [17]. Even in rodent models carrying genetic risk factors associated with human stroke, e.g. insulin-dependent genetic diabetes [18], non-insulin dependent diabetes [19] and hypertension [10], females are less sensitive to cerebral ischemia than males. However, it should be noted that the majority of these studies have been carried out in rodents that are young (3–4 months) or middle aged (12–14 months). Whether sex differences reverse with age is not clear, although one recent study suggests that ischemic damage increases with age in female, but not male, mice [20]. Nevertheless, the data in aggregate emphasize that sex shapes outcome in experimental brain injury models, mimicking the epidemiology of human stroke.

3. XX vs. XY cells respond differently to injury

In vitro data directly support the related concepts that cell death after injury is sexually dimorphic and that some molecular injury and survival mechanisms are sex-specific. The latter concept has been formulated by studies of male vs. female cell cultures grown without background steroids. In early observations, female dopaminergic neurons were shown to tolerate exposure to toxic dopamine concentrations and survive twofold relative to male cells [21]. Similarly, female neurons from the cortical plate or ventricular zone have greater longevity in culture than male cells, and differentially express higher levels of phosphorylated kinases such as Akt [22]. Sensitivity to glutamate, peroxynitrate (ONOO) and staurosporine in neuronal culture is sex-specific, with male neurons being more susceptible to glutamate and ONOO than females. In contrast, response to oxidants such as hydrogen peroxide (H_2O_2) is independent of cell sex [23].

These observations are not limited to neurons. Similar sex specificity is present in the astrocytic response to oxygen and glucose deprivation (OGD), an *in vitro* model of "ischemia", and to toxins that stimulate cell death pathways. Astrocytes are important supportive cells for neurons and brain vascular components in normal brain functioning. The inflammatory response of astrocytes and other glial cells, known as reactive gliosis, is a key component of the brain's response to injury and neurodegenerative conditions. In cortical astrocytes, cultured from rat pups and segregated by sex, we have observed that female astrocytes are more resistant to OGD as compared to male cells, but sustain greater cell death when inflammatory mediators are combined with OGD compared to OGD alone [24]. Female astrocyte resistance to OGD is, in part, mediated by their ability to engage the endogenous enzyme P450 aromatase, metabolize 17-β estradiol from androgen precursors, and capitalize on estradiol's cytoprotective properties [25]. To verify the importance of sex differences in P450 aromatase function after OGD, we developed a novel method to establish sex-specific and genotype-specific single pup primary astrocyte cultures from wild-type and aromatase knockout (ArKO) mice [25]. Using this technique, we showed that female astrocytes lacking the P450 aromatase gene were highly sensitive to OGD, unlike their wild-type counterparts.

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Table 2
Androgen effects in brain injury models.

Cell/species	Insult/androgen treatment	Effect	References
Murine hippocampal cell line (HT22)	Glutamate exposure for 24 h/T was added immediately before the addition of glutamate	T at 10 μM exacerbates neuronal cell death induced by glutamate toxicity	[46]
Primary rat oligodendrocytes	Brief overactivation of AMPA or kainate receptors for 15 min/T was added 24 h before AMPA/kainate receptor overactivation	T at 100 nM enhances both AMPA and kainate toxicity, and damaging effects are prevented by AR antagonist flutamide, but not aromatase inhibitors	[47]
Mixed mouse cortical cultures containing both astrocytes and neurons	NMDA exposure/T was applied daily during the 4 days preceding the NMDA pulse	T amplifies excitotoxic neuronal death at 10 μM, whereas it is protective at 10 nM and inactive at intermediate concentrations. Low concentrations of T become neurotoxic in the presence of aromatase inhibitors	[48]
Primary rat cerebellar granule cells	Oxidative stress induced by hydrogen peroxide exposure for 24 h/T was added 48 h before the induction of oxidative stress	T at 1 μM protects granule cells from hydrogen peroxide induced cell death, which is abrogated by AR antagonist flutamide	[49]
Primary rat hippocampal neurons	Exposure to toxic concentrations of aggregated Aβ for 48 h/T or DHT was added 24 h before Aβ exposure and retained throughout experiments, or only during 24 h pretreatment period or only during Aβ exposure	T and DHT at 1–100 nM protect neurons from Aβ-induced cell loss when presented to cells throughout experiments or simultaneously with but not prior to Aβ exposure	[50]
Primary rat hippocampal neurons	Exposure to toxic concentrations of aggregated Aβ for 24 h/T or DHT was added 2 h before and during Aβ exposure	T and DHT at 10–100 nM protect neurons from Aβ-induced cell loss	[51,52]
Primary human cortical neurons	Microinjection of Aβ into neurons/T was added immediately or 1 h before injection and further incubated for 24 h	T at 10 nM protects neurons from Aβ-induced cell loss. T-mediated neuroprotection is blocked by flutamide	[53]
Primary human neurons	Serum deprivation/T was added during serum deprivation	T at 4 nM reduces apoptosis between 24 and 96 h after serum deprivation. Flutamide, but not aromatase inhibitors, prevents T-mediated neuroprotection	[54]
Primary mouse cortical astrocytes	Oxidative stress induced by exposure to IAA for 12 h/T was added during IAA exposure	At 10 μM, DHT protects astrocytes, while cell membrane impermeable DHT–BSA conjugates enhance cell death induced by IAA	[55]
Young adult male mouse	Focal ischemia/Mice were castrated and implanted with different doses of T and DHT 1 week before MCAO	Low doses of T and DHT protect against, while high doses exacerbate ischemic damage at 24 h and 9 days reperfusion	[45]
Young adult male rat	Focal ischemia/castrated males were subcutaneously implanted with T at 2 days and T pellets were removed at 1, 2, 4 and 6 h before MCAO	Removal of T pellets from castrates at 6 h before MCAO decreases infarct sizes at 24 h reperfusion compared to animals with physiological testosterone	[46]
Young adult male rat	Focal ischemia/male rats were castrated and implanted with T 1 week before MCAO	A strong positive correlation between plasma testosterone concentrations and ischemic lesion sizes is observed at 24 h reperfusion	[56]
Young adult male rat	Focal ischemia/castrated rats received subcutaneous implantation of T on day 7 post-MCAO	T does not alter infarct volumes but improves neuronal deficits at 3 weeks after implantation	[58]
Young adult and middle aged rat and mouse	Focal ischemia/young adult animals were castrated and implanted with physiological doses of T and DHT 1 week before MCAO. Intact aged animals were not castrated but implanted with physiological doses of T and DHT 1 week before MCAO	At 22 h reperfusion, T and DHT protect gonadally intact middle aged males, while exacerbate ischemic damage in young adult castrates	[59]
Young adult male rat	Focal ischemia/male rats were castrated and implanted with DHT 1 week before MCAO	High but physiologically relevant doses of DHT increase infarct volumes in castrated males at 22 h reperfusion	[60]

Abbreviations—AMPA: α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; Aβ: amyloid beta; BSA: bovine serum albumin; DHT: dihydrotestosterone; IAA: iodoacetic acid; MCAO: middle cerebral artery occlusion; NMDA: N-methyl-D-aspartic acid; T: testosterone.

Furthermore, sex differences in astrocytic sensitivity to OGD were largely absent in ArKO cells, confirming that aromatase and sex-specific hormone production is an important basis for astrocytic sexual dimorphism under injury conditions.

4. Sex-specific ischemic cell death mechanisms

Emerging evidence suggests that the molecular signaling pathways engaged by cerebral ischemia *in vivo* or by cytotoxin administration to cultured cells are not identical in male vs. female brain. Data from genetically engineered mouse strains established the current working hypothesis that sexual dimorphism in ischemia is partly due to the genetic complement of cells, rather than solely to hormonal environment. When both sexes of genetic knockout mice are studied, one can readily observe if the gene of interest acts in an overtly sex-dependent way. Several genetic mechanisms not linked to sexual development or function have been shown to act dimorphically under injury conditions (Table 1). For example, neuronal nitric oxide synthase (nNOS) is well known to play an important role in neuronal death by fueling nitric oxide toxicity, peroxynitrite formation and protein nitration. Genetic deletion or pharmacological inhibition of nNOS is neuroprotective in the male, but not female, brain [14] or in hippocampal slice preparation [26].

To date, one of the best studied sex-specific mechanisms involves a set of molecules that lead to neuronal apoptosis after ischemic injury. Apoptosis, originally referred to as programmed cell death, is a slower form of post-ischemic pathology than frank tissue necrosis and so has received considerable interest as part of the search for human neuroprotective therapies. Apoptotic

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pathology involves at least two signaling cascades, one initiated through intrinsic, mitochondria-mediated mechanisms involving cytochrome *C* release, apoptosome assembly and caspase cleavage, and hence is known as a "caspase-dependent" pathway. An alternative caspase-independent pathway is triggered by post-ischemic DNA damage and has been recently named "parthanatos" (for recent review [27]). This caspase-independent pathway involves the activation of poly-ADP ribose polymerase (PARP), release of apoptosis-inducing factor (AIF) from the mitochondria and translocation of AIF to the nucleus to induce chromatin condensation, and large scale DNA fragmentation. PARP is a DNA repair enzyme that is critical to the caspase-independent death signaling that occurs after excitotoxic or ischemic insults in male mice or mixed sex cell culture [28]. Genetic deletion or pharmacological inhibition of PARP improves brain outcomes from cerebral ischemia in males [28], but not in females regardless of ovarian hormone status [14]. Similar sex-specificity has been reported in the developing brain treated with hypoxia–ischemia [29,30]. These early observations provided initial clues that the PARP-AIF death pathway may be highly engaged in male ischemic brain, less so in the female. Subsequent work has confirmed and expanded our understanding that the nNOS-PARP-AIF pathway is an important target in male cerebral ischemic pathology, while the intrinsic, caspase-dependent pathway is vital to female neuronal death. The therapeutic implication is that therapy designed to block nNOS/PARP mechanisms are likely to benefit the male, while anti-caspase therapies will be most effective in the female. Recent evidence in animals is bearing out this hypothesis (Table 1 and Refs. [30–34]).

In summary, these findings suggest that the response to cerebral injury *in vivo* and *in vitro* is partially a function of the sex of the cell. Examples of molecules that have sexually dimorphic roles in cerebral ischemic cell death mechanisms include apoptosis-inducing factor [30], caspase 3 [31,32], PARP [14,26,29], NOS [14,35], glutathione [23], Akt [36], astrocytic aromatase [24,25], glial fibrillary acidic protein (GFAP) [37], angiotensin II type 2 receptor [38], and the soluble epoxide hydrolase (sEH) [39]. However, this in no way discounts the importance of gonadal steroids or brain-derived neurosteroids as modulators of oxidant, toxic and ischemic challenges to the brain. An important, but highly understudied, issue is how the male "ischemic-sensitive" phenotype is established and if androgens contribute to the sexual dimorphism of ischemic brain injury.

5. Androgens and cerebral ischemia

Consistent but sparse evidence suggests that male sex and androgens impact ischemic outcomes and mechanisms of brain damage [7,40]. It has been assumed that androgens are detrimental to ischemic pathobiology because (1) male sex is a known stroke risk factor and (2) male animals sustain greater histological damage after experimental stroke than females. However, low circulating testosterone levels have also been associated with higher stroke incidence and worse outcomes after stroke in men [41–44]. Importantly, androgen levels dramatically drop following both experimental and clinical stroke [44,45]. Thus, ischemia-induced androgen loss may be as important as the steady level of androgens prior to the ischemic insult. In bench studies that control androgen levels, the data are conflicting and indicate that androgens can protect or exacerbate ischemic damage. Moreover, our recent finding that androgens exhibit highly dose-dependent effects on ischemic outcomes in animals further complicates the issues that surround male sex steroids [45]. Table 2 summarizes available *in vitro* and *in vivo* data on androgen effects in various brain injury models.

6. *In vitro* effects of androgens

The brain is a target tissue for androgens, and numerous *in vitro* studies have suggested that androgens have direct effects on the responses of neurons and glia to injury. These insults include oxidative stress, excitotoxicity, serum deprivation and amyloid β (Aβ) exposure. In aggregate, androgens can exacerbate or protect against damage from these insults *in vitro*. For instance, testosterone increases glutamate neurotoxicity in HT22 neuronal cells at 10 μM [46] and amplifies AMPA/kainate receptor associated oligodendrocyte damage at 100 nM [47]. Another recent study demonstrates that testosterone protects against or exacerbates damage from excitotoxicity in cultured primary neurons, depending on the steroid concentration. Testosterone at supra-physiological concentrations (10 μM) amplifies excitotoxic neuronal death induced by N-methyl-D-aspartic acid (NMDA), however it is protective at 10 nM and has no effect at intermediate concentrations [48]. In this *in vitro* model, the neuroprotective testosterone acts indirectly, i.e. via aromatization into 17β estradiol. In the presence of aromatase inhibitors, testosterone shows toxicity. In other studies, testosterone directly protects cultured neurons, as does the potent non-aromatizable androgen receptor (AR) agonist, dihydrotestosterone (DHT). For example, 1 μM testosterone protects cultured cerebral granule cells from oxidative stress, presumably through AR signaling since protection is abolished by flutamide [49]. In cultured primary hippocampal neurons, both testosterone and DHT (1–100 nM) protect against Aβ-induced cell death, and protection is not attenuated by aromatase inhibition [50–52]. These results are consistent with an earlier study showing that both testosterone and estradiol confer neuroprotection to human cortical neurons from toxic Aβ peptide. Interestingly, the protection by androgens and estrogen is mediated by AR and estrogen receptor, respectively [53]. Testosterone (4 nM) also protects human neurons from serum deprivation-induced apoptosis via androgenic signaling [54]. One interpretation of the apparent paradox of beneficial vs. detrimental effects of androgens is that two potentially competing signaling mechanisms are engaged *in vitro*. For instance, in cultured astrocytes, activation of a putative membrane-associated AR by DHT promotes cell death, while signaling via classic intracellular AR is associated with neuroprotection [55]. Clearly, further study is required to unravel the mechanisms underlying the shift between androgen protective and damaging effects. Nevertheless, currently available *in vitro* studies have suggested that androgens can exhibit neuroprotective effects mechanistically independent from those of estrogen signaling.

7. Androgen actions *in vivo*

Consistent with the field's initial assumption that androgens play a deleterious role in cerebral ischemia, some studies show that androgen treatment in castrated male rodents increases histological damage [46,56]. Stressors that reduce testosterone levels, e.g. anesthesia administered before the onset of cerebrovascular occlusion, improve ischemic outcomes in the male [57]. Yet, androgens administered after experimental stroke accelerate functional recovery after stroke [58]. This latter finding is consistent with clinical data showing that low plasma testosterone in men is inversely associated with stroke severity, infarct size and functional recovery [43]. Similarly, lower testosterone is associated with higher incidence of stroke in men [41]. In contrast to the assumed deleterious role of androgen, these studies support the hypothesis that androgens can be neuroprotective in cerebral ischemia. We have recently shown that maintaining testosterone or DHT plasma levels within the low physiological range throughout an episode of focal cerebral ischemia confers protection to both adult castrates (Fig. 1) and gonadally intact aged animals with naturally declining andro-

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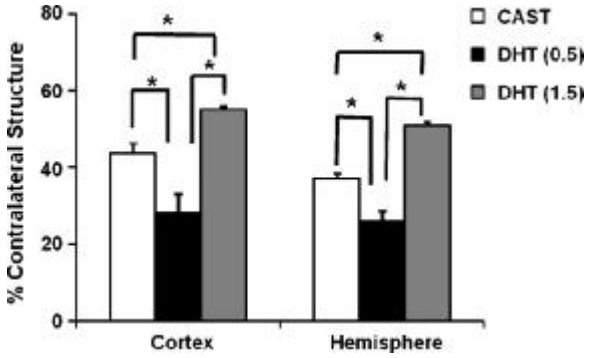

Fig. 1. Dose-dependent effects of DHT in experimental stroke. Low physiological doses of DHT decrease, while high but physiologically relevant doses of DHT increase, infarct volumes in young adult castrated male mice following middle cerebral artery occlusion. Infarct volumes are expressed as percentages of contralateral structures in castrated mice (CAST, $n = 20$) and in castrates implanted with 0.5 mg DHT [DHT(0.5), $n = 11$] or 1.5 mg DHT [DHT(1.5), $n = 12$]. Values are mean ± SEM. $^*P < 0.05$. Adapted from data as published in Ref. [45].

gens [45,59]. The benefit of controlled androgen availability has now been demonstrated in both rats and mice, and is mediated via direct AR signaling since neuroprotection is abrogated by flutamide [45]. Using the same hormone implantation technique, we also observed that high but physiologically relevant androgen levels exacerbate ischemic damage in castrated males [45,60], suggesting that androgens and androgen availability also have complex dose-dependent effects *in vivo*. To evaluate potential mechanisms behind androgens' ability to enhance ischemic damage, we used microarray and real time PCR to identify gene candidates induced by DHT [60]. At high but physiologically relevant doses, DHT enhanced pro-inflammatory gene expression after middle cerebral artery occlusion, suggesting that enhanced inflammation may contribute to the deleterious effects of DHT. (For full list of genes identified by Affimetrix microarray in DHT treated castrated rats, see Ref. [60].)

8. Neuroprotective mechanisms of androgens

Despite the complexities of androgenic dose–response relationships in ischemia, their neuroprotective properties remain of interest. AR expression has been confirmed in neurons throughout the brain, including cortical and striatal regions impacted by our focal ischemia models [61,62], therefore AR-regulated transcription is a potential mechanism underlying androgen neuroprotection. Although definitive characterization of genes that participate in androgen's neuroprotection has not yet been accomplished, antioxidant proteins and stress-induced heat shock protein HSP70 have been implicated [49,53,63–65]. For example, testosterone protects cerebral granule cells against oxidative stress in an AR-dependent manner associated with a twofold increase in catalase activity [49]. Androgens also increase HSP70 expression after Aβ induced neurotoxicity [53]. Androgen non-genomic, rapid signaling pathways also have been implicated in androgen neuroprotection in cerebral ischemia. For example, androgen-induced activation of Akt protects cortical astrocytes against oxidative stress [55] and activation of mitogen-activated protein kinases (MAPK) protects hippocampal neurons against cell death induced by Aβ neurotoxicity [51]. In astrocytes and glial cell lines, DHT protection is associated with the activation of phosphoinositide-3 kinase (PI3K)/Akt as well as MAPK, while cell membrane impermeable DHT–BSA conjugates suppress MAPK and Akt activation and increase cell death [55,66]. These data suggest that intracellular AR-dependent activation of protective kinase signaling is important to DHT's actions. Lastly, recent studies emphasize that androgens can rapidly but sustainably activate cAMP response element binding protein (CREB) via non-genomic pathways both in neuronal and non-neuronal tissues [67–70]. Collectively, these results implicate a possible role of androgen activated non-genomic pathways in androgen neuroprotection following cerebral ischemia. Continued investigation of non-genomic signaling may provide important insights into the mechanisms underlying androgen neuroprotection.

In summary, androgens are clearly important but currently understudied steroid actors that are relevant to stroke and may advance our understanding of male vulnerability to cerebrovascular insults. Despite the complexity of androgen actions *in vitro* and *in vivo*, current clinical observations and limited animal data suggest that physiological levels of androgens exert neuroprotection in the male brain. The recently identified dose-dependent effects of androgens and the potential importance of ischemic stress-induced androgen loss may account for some controversial results in experimental stroke. Whether deleterious or beneficial, it is already clear that pleiotrophic androgens will impact multiple cell types of the injured brain and act through multiple molecular pathways in cerebral ischemia.

9. Sex on the brain in stroke: an opportunity

In conclusion, accumulating evidence strongly suggests that biological sex and sex steroids shape both the outcomes of cerebral ischemia and attendant cell death mechanisms. Understanding the differences between male and female cell responses to injury and repair offers an enormous opportunity to design and refine our therapeutic targets in a manner beneficial to both sexes. In addition, the use of animal and cell models that allow determination of sex differences, as well as the sex-specific scaffolding on which steroids operate, will allow us to understand experimental data in novel ways. Instead of minimizing or excluding sex and sex steroids, as is frequently done in translational disease research, there is much to be gained by elucidating such fundamental mechanisms.

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