Androgens and Stroke: Good, bad or indifferent?

Nidia Quillinan[1], **Guiying Deng**[2], **Himmat Grewal**[1], and **Paco S. Herson**[1,2]

[1]Department of Anesthesiology, University of Colorado Denver, Anschutz Medical Campus, 12800 E. 19th Ave., Aurora, CO 80045

[2]Department of Pharmacology, University of Colorado Denver, Anschutz Medical Campus, 12800 E. 19th Ave., Aurora, CO 80045

Abstract

Cerebral ischemia caused by loss of blood supply to the brain during cardiac arrest or stroke are major causes of death and disability. Biological sex is an important factor in predicting vulnerability of the brain to an ischemic insult, with males being at higher risk for cardio-cerebrovascular events than females of the same age. However, relative incidence of stroke between the genders appears to normalize at advanced ages. Therefore, many scientists have focused on the mechanisms of sex differences in outcome following brain ischemic injury, with a particular emphasis on the role of sex steroids. The majority of studies indicate that female sex steroids, such as estrogen and progesterone, play important roles in the relative neuroprotection following cerebral ischemia observed in females. However, less is known about male sex steroids and brain damage. This review describes the state of our knowledge of androgen-related contributions to neurological injury and recovery following cerebral ischemia that occurs following stroke. Experimental studies examining the effects of castration, androgenic agonists and antagonists and aging provide valuable insights into the role of androgens in clinical outcome following cerebrovascular events.

Introduction

Stroke is a leading cause of death and disability in the United States and clinical literature readily recognizes male gender as a significant risk factor. Experimental stroke models recapitulate the robust epidemiological gender differences in prevalence, demonstrating gender differences in tissue (histological) outcomes. The experimental stroke field has focused largely on the role of female sex steroids, such as the estrogens (for review see (McCullough & Hurn, 2003; Herson *et al.*, 2009; Lebesgue *et al.*, 2009; Liu *et al.*, 2010). The topic of the current review is the role of androgens in shaping the male brain response to

Corresponding Author: Paco S. Herson, PhD, [1]Department of Anesthesiology, University of Colorado School of Medicine, 12800 E. 19th Ave., Aurora, CO 80045, [2]Department of Pharmacology, University of Colorado School of Medicine, 12800 E. 19th Ave., Aurora, CO 80045, paco.herson@ucdenver.edu, Telephone: (303) 724-6628, Fax: (303) 724-1761.

ischemic injury. Emerging experimental studies indicate that male sex steroids, or androgens (testosterone or dihydrotestosterone; DHT), can have both deleterious and protective effects on the central nervous system (CNS) following cerebrovascular insults such as stroke or cardiac arrest (Cheng *et al.*, 2007; Uchida *et al.*, 2009; Nakano *et al.*, 2010; Cheng *et al.*, 2011). Levels of circulating androgens change with normal development and aging and these changes may contribute to vulnerability of the male brain to an ischemic event. Correlation between testosterone levels and incidence of cerebrovascular disease has been reported, with low plasma testosterone in aging men being associated with increased risk for cardiovascular disease and stroke. In contrast, androgens in the young may contribute to injury, such that increased levels of androgens in young adults increases ischemic injury. This complex age-dependent and dose-dependent role of androgens in incidence and vulnerability of males to ischemic injury is discussed in the current review. The combination of clinical and basic science literature leads to the conclusion that while high levels of androgens enhance ischemic brain injury, the natural decline in physiological levels of androgens contributes to increased incidence and worsened outcome with aging. Therefore, it appears the most logical approach to take clinically would be to attempt to maintain men within the normal physiological range of androgens throughout the lifespan.

Androgens and stroke incidence

Ischemic stroke and cardio/cerebrovascular disease (CVD) are multifactor diseases that involve several predisposing risk factors that contribute to incidence and severity of injury. Male sex is a recognized risk factor for stroke, albeit non-modifiable. Indeed, several retrospective studies examining gender differences in stroke incidence have demonstrated that males are at a higher risk of stroke across most of the lifespan (Hollander *et al.*, 2003; Rothwell *et al.*, 2005; Reeves *et al.*, 2008; Appelros *et al.*, 2009). The relatively high levels of testosterone in young adult men may contribute to increased incidence of cerebrovascular disease, as shown by the South London Stroke register which shows males have a higher incidence of stroke in the young adult population (15–54y; 58.5%) (Wang *et al.*, 2013). Interestingly, the incidence of stroke between males and females are similar in the >54y population (Wang *et al.*, 2013). Similarly, the large North Manhattan Stroke Study observed that stroke rates in men and women equalized at older ages (Sacco, 1998; Reeves *et al.*, 2008). The gender differences that are observed in young adulthood are generally attributed to the protective role of estrogens in female, which reduce dramatically following menopause (McCullough & Hurn, 2003). However, the relatively high levels of androgens in young adult males likely also contribute to increased vulnerability. Clinical evidence for the detrimental role of androgens in ischemic injury is difficult to obtain, however testosterone supplementation has been used for some purposes, such as the treatment of androgen deficiency in men or to improve performance in athletics. Testosterone levels in men may contribute to the relatively increased incidence of stroke via altering various known risk factors of stroke. Specifically, anabolic steroid use is associated with cardiovascular pathologies including increased vascular tone/arterial tension and promoting platelet aggregation (Littleton-Kearney & Hurn, 2004). There are several case reports observing stroke and sudden cardiac arrest in otherwise healthy young men with a history of

anabolic steroid abuse (Fineschi *et al.*, 2007; Youssef *et al.*, 2011). However, anabolic steroid use and incidence/severity of stroke remains to be determined in large scale studies.

Aging and loss of androgens

The role of endogenous levels of androgens in determining incidence and severity of injury points to a protective role of androgens, such that acute and chronic (aging) decreases in circulating levels of androgens increase incidence of stroke and likely worsen outcome. There are several studies that indicate that lower testosterone levels in young and older males is associated with increased cardiovascular risk factors and stroke incidence (Muller *et al.*, 2004; Yeap *et al.*, 2009; Chock *et al.*, 2012; Firtser *et al.*, 2012). While the mechanism of increased CVD incidence remains unclear, evidence indicates that low testosterone in men is associated with increased blood glucose level and metabolic syndrome, which may predispose men to higher risk of CVD and mortality (Haffner *et al.*, 1994; Kaplan *et al.*, 2006; Kapoor & Jones, 2008; Kupelian *et al.*, 2008). Similarly, acute androgen removal, such as occurs during androgen deprivation therapy (ADT) in prostate cancer patients increases risk of stroke. Azoulay L. et al. prospectively studied 22,310 prostate cancer patients for a mean of 3.9 years and demonstrated that the risk of stroke/TIA are increased with all types of ADTs, with the highest risk observed in patients who underwent bilateral orchiectomy (Azoulay *et al.*, 2011). Moreover, ADT was associated with an increased incidence of diabetes and other cardiovascular-related risk factors, which may increase stroke risk and increase ischemic injury (Taylor *et al.*, 2009; Bain, 2010). Thus, ADT provides a unique insight into the role of androgen removal on ischemic brain injury, indicating that removal of endogenous androgens appears to be detrimental. There is evidence that testosterone therapy is beneficial for overall quality of life in young men (<50 years) with low testosterone levels and has been shown to reduce mortality (Shores *et al.*, 2012; Spitzer *et al.*, 2013). However recent studies in old men or those with a history of cardiovascular disease which demonstrate increased mortality and stroke incidence in patients receiving testosterone therapy (Basaria *et al.*, 2010). In summary of the adult clinical literature, androgens are capable of both detrimental and beneficial effects and that maintaining androgen levels within the young adult 'normal' physiological range is likely the optimal condition to minimize male risk for stroke. Future studies are needed to characterize the benefits and risks of testosterone therapy on cerebrovascular health in in the middle and old-aged populations and with comorbidities. The cellular and molecular mechanisms of these contradictory roles of androgens in ischemic injury are discussed further below.

Androgens and childhood stroke

Childhood stroke, while relatively rare, has devastating consequences on quality of life. The level of androgens varies throughout life, thus it is likely that androgen related effects on brain ischemia would vary with development and age. Incidence of ischemic stroke is higher in neonates and infants compared to older children of the same gender (Fullerton *et al.*, 2003; Steinlin *et al.*, 2005; Lo *et al.*, 2009). In general, androgen levels are very low in pre-pubertal children and likely have little impact on stroke risk. Neonates and infant boys are the exception, however, as there are two postnatal surges in testosterone levels that occurs at

1 week and 4–6 months after birth (McIntyre, 2006; Hill, Threlkeld, *et al.*, 2011; Hill & Fitch, 2012). Therefore, it is likely that androgen-related effects following brain ischemia is more influential to outcome during these periods. Consistent with this hypothesis, recent studies demonstrate increased incidence of stroke in boy neonates and infants (<4 years old) compared to girls of the same age (Fullerton *et al.*, 2003; Golomb *et al.*, 2003; 2009; Lo *et al.*, 2009). During the pre-pubertal adolescent ages (4–15) no gender differences are observed (Lo *et al.*, 2009) when females have a slightly higher level of circulating androgens (Courant *et al.*, 2010). In contrast, the male predominance in stroke incidence is evident in children over 15, after the onset of puberty (Lo *et al.*, 2009). These data implicate relative levels of androgens in enhancing the rate of stroke in boys, as the ages associated with increased levels of testosterone (infants and post-pubertal) are the ages associated with increased risk of stroke compared to girls. However, viewed in light of the adult literature which indicates that androgens in young adults are likely beneficial, it is equally possible that the post-pubertal effect is mediated by an increase in protective female steroids. Nonetheless, the enhanced vulnerability in neonates and infants (<4 years) is an important link between ischemic insult and androgens. Research scientists have focused on this enhanced risk of childhood stroke in neonates and children in their first years of life by taking advantage of animal models of neonatal stroke. Studies using neonatal animal models indicate that male neonates exhibit greater long-term deficits following injury than age matched females, including increased brain volume loss, enhanced dysmyelination and increased behavioral deficits following hypoxia and hypoxic-ischemic brain injury (Mayoral *et al.*, 2009; Hill, Alexander, *et al.*, 2011; Hill, Threlkeld, *et al.*, 2011; Lan *et al.*, 2011). Similar to the epidemiological data described above for 4–15 year old pre-pubertal children, a mouse model of pediatric stroke demonstrated equivalent injury in male and female pediatric mice following MCAO (Herson *et al.*, 2013). These observations are consistent with a role for androgens in sensitizing the male brain to ischemic injury as neonates/infants, which is lost as androgen levels decline during early childhood.

Animal Models to assess the role of androgens in acute injury

While patient studies can provide some insight into androgens as risk factors for stroke and cardiovascular disease, there is very little known in the clinical population regarding what impact androgen levels have on infarct size and outcome in those patients that do have an ischemic brain injury. Animal models of cerebral ischemia have been used extensively to examine the role of sex steroids in gender differences in neurological injury. Animal models of cerebral ischemia reliably demonstrate that male animals suffer larger ischemic injury compared with age-matched females (Hall *et al.*, 1991; Liu *et al.*, 2009; Manwani *et al.*, 2013). Extensive literature links estrogen to the relative protection observed in females (For review see (McCullough & Hurn, 2003; Herson *et al.*, 2009; Lebesgue *et al.*, 2009) and is the focus of an accompanying article in the issue. However, the effects of androgens in cerebral ischemia are controversial and remain understudied. Testosterone is the most abundant male sex steroid and generally the focus of clinical investigation. However, experimentally the use of testosterone is complicated by the fact that testosterone can be converted into estrogen by the enzyme aromatase (Roselli *et al.*, 2009). To avoid this possible complication, experimental studies generally use the potent metabolite of

testosterone, 5α-dihydrotestosterone (DHT) which binds with high affinity to the androgen receptor and is not able to be converted by aromatase to estrogen. The mechanistic studies described below have ruled out the possible confounding effects of aromatase by either using DHT experimentally or inhibiting aromatase. Thus, for the purpose of the current review we focus only on studies that have directly implicated androgen signaling in the effects reported. Multiple studies using the middle cerebral artery occlusion (MCAO) model of focal ischemic stroke have examined the effects of androgen removal and replacement in cerebral infarction. The majority of studies focused on the role of androgens in ischemic outcome demonstrate that castration of young males to remove endogenous androgens prior to MCAO reduced infarct size and androgen replacement increases infarct to levels similar to that observed in hormonally intact males (Yang *et al.*, 2002; Cheng *et al.*, 2007; 2009; Uchida *et al.*, 2009; Vagnerova *et al.*, 2010). These studies led to the conclusion that androgens enhance injury in male animals (Table 1). However, careful dose-response relations of androgen replacement prior to MCAO indicates that low doses of androgens (testosterone or DHT) further reduces infarct size compared to castration alone and that high doses exacerbate infarcts (Uchida *et al.*, 2009). The dose-dependent effects on outcome, coupled with fluctuating androgen levels across the lifespan make it difficult to define a simple role for androgens in injury.

Recent studies suggest that the age-dependent decline in circulating androgens is associated with worsened outcome in aging mice following MCAO (Liu *et al.*, 2009) and androgen replacement is protective in middle aged mice (Cheng *et al.*, 2009). Thus, experimental models of stroke demonstrate that androgens are capable of playing both a protective and damaging role in ischemic injury. Most importantly, data obtained from young adult male mice does not reliably model the human data; that is removal of androgens appears to be detrimental in people and beneficial in experimental animals. In contrast, data obtained using middle aged and aging animals more faithfully model the clinical situation. Therefore, it is strongly recommended that future studies aimed at unraveling the role of androgens in stroke outcomes take advantage of aging animals to provide more reliable pre-clinical data.

Mechanisms of androgen influence on ischemic injury

The deleterious and protective effects of testosterone are predominantly androgen receptor dependent (Cheng *et al.*, 2007; Uchida *et al.*, 2009; Nakano *et al.*, 2010). Canonical androgen signaling involves androgen binding to cytoplasmic androgen receptors, translocation to the nucleus and transcription of androgen responsive genes (for review see (Bennett *et al.*, 2010)). Recent evidence is emerging that androgens interact with rapid signaling pathways, although the presence of a membrane associated androgen receptor remains controversial. Nonetheless, extensive evidence exists demonstrating that androgens interact with a variety of signaling pathways involved in cell death and survival, thereby exhibiting both neurotoxic and neuroprotective effects (Figure 1). Several mechanisms have been identified by which androgens increase injury. Exogenous testosterone can increase glutamate-induced calcium influx, thereby increasing excitotoxic injury in cultured hippocampal, oligodendrocyte and glial cells (Caruso *et al.*, 2004; Foradori *et al.*, 2007; Holmes *et al.*, 2013). Similarly, in immature neuronal cultures which use GABA as an excitatory neurochemical, testosterone increases GABA induced calcium influx and injury

(Nuñez & McCarthy, 2008). Testosterone was also observed to induce pro-apoptotic p53 target genes (Bax, MDM2, p21) in cultured oligodendrocytes (Caruso *et al.*, 2004). A number of androgen gene targets have been identified in mouse brain following MCAO that likely increase injury through increased inflammation, dysregulation of the blood brain barrier or altered cell signaling (Cheng *et al.*, 2007). The use of BSA conjugated testosterone and DHT have revealed the possibility of a non-classical membrane-associated androgen receptor that decreases cell survival, causing a reduction in ERK and AKT phosphorylation (Nguyen *et al.*, 2005; Gatson *et al.*, 2006; Gatson & Singh, 2007).

In contrast, several studies have demonstrated that androgens can be neuroprotective (Ahlbom *et al.*, 2001; Nguyen *et al.*, 2005; Pike *et al.*, 2008). Interestingly, ERK and AKT appear to be differentially phosphorylated in a concentration and agonist-dependent manner, with low doses of androgens increasing phosphorylation and promoting cell survival (Nguyen *et al.*, 2005; Gatson *et al.*, 2006; Gatson & Singh, 2007). Other pathways which may contribute to the neuroprotective properties of androgens include increased antioxidant catalase activity, increased expression of salt induced kinase 1, and CREB activation (Ahlbom *et al.*, 2001; Nguyen *et al.*, 2009; Cheng *et al.*, 2011). Figure 1 is a schematic illustrating the various signaling pathways observed to be modified by androgens in the context of injury. Similar to the observations made in experimental animal models of stroke, signaling studies demonstrate that androgens can exert opposing effects on cell survival. The relative balance of each of these signaling pathways is likely determined by the timing and dose of androgen exposure as well as the specific cell type and differential activation of intracellular versus membrane associated receptors.

In addition to the role of sex steroids in determining ischemic sensitivities, emerging data indicates that hormone-independent sex differences in cell death signaling exists. The majority of these data are obtained using primary cell cultures obtained from embryos grown in the absence of sex steroids and then exposed to various injurious stimuli. Several studies have converged to provide the overarching observation that male cell death after ischemia-reperfusion injury is mediated by oxidative stress, subsequent over-activation of poly(ADP)ribose polymerase (PARP) and activation of transient receptor potential M2 (TRPM2) cation channels (Figure 1), while female cell death is predominantly mediated by apoptosis involving caspase activation (petere *et al.*, 2008). However, recent work has begun to consider the influence of androgens on the PARP-TRPM2 cell death pathway in adult animals. It appears that early development provides a sex-stratified signaling foundation such that male-specific ischemic injury is mediated by over-activation of PARP and TRPM2, however in adulthood the presence of androgens adds an additional level of complexity. Specifically, castration of adult mice reverses ischemia-induced activation of PARP (Vagnerova *et al.*, 2010) and TRPM2 (Shimizu *et al.*, 2013) and thus alters cell death mechanisms. Thus, sex-specific cell death pathways laid down early in development are modified during adulthood by the presence of circulating sex steroids.

Chronic androgen treatment and recovery

Clinical data demonstrates that serum testosterone levels are reduced in patients following stroke and reduced testosterone levels are associated with poor outcome in men (Dash *et al.*,

1991; Jeppesen *et al.*, 1996). Animal studies have primarily focused on the effects of pre-stroke levels of androgens on acute injury, with less attention paid to whether manipulating post-stroke levels of androgens will alter the rate of recovery and long-term outcomes. An exception to this is the work from Pan et al, which examined whether delayed testosterone replacement (7 days after MCAO) in castrated males altered functional recovery compared to androgen deficient males (Pan *et al.*, 2005). This study observed that testosterone-treated males had accelerated recovery of motor function compared to testosterone deficient castrated controls. In addition, there are a few studies that point towards a role for delayed and chronic androgen replacement as a potential restorative therapy following stroke. For example, testosterone replacement after stroke has been associated with less reactive astrocyte hypertrophy in peri-infarct areas, a process that can prevent cortical remodeling (Moon *et al.*, 2000; Chen *et al.*, 2002; Tan *et al.*, 2005). The critical role testosterone plays on neuronal viability during development and its impact on neurogenesis, axonal sprouting and plasticity make it likely that low testosterone following stroke would be detrimental to the repair process following injury. The role of androgens in post-ischemic neurogenesis was examined in intact, castrated and androgen treated mice and revealed that endogenous androgen receptor signaling did not alter post-ischemic neurogenesis, but that supraphysiological androgen levels suppress neurogenesis after stroke (Zhang *et al.*, 2013). Effects of testosterone depletion and replacement on neuronal structure and function have been examined in rodents, although not in the context of brain injury. Therefore, consideration of the role of androgens in determining acute ischemic injury as well as chronic repair and plasticity should be considered.

In addition to plasticity and repair in the CNS following cerebral ischemia, alterations in immune function is a major contributor to morbidity and long-term recovery after stroke. Thus, it may be important to consider the impact of testosterone levels on immune function following brain injury, particularly following stroke. Clinical studies have shown that peripheral immunosuppression is a major consequence of stroke and can increase susceptibility of patients to a secondary infection, resulting in longer hospital stays and poorer prognosis of recovery. Indeed, fatal infection is the most common cause of death in patients who survive the acute phase of stroke (Meisel *et al.*, 2005; Chamorro *et al.*, 2007; Dirnagl *et al.*, 2007). Animal studies have demonstrated post-stroke peripheral immunosuppression, characterized by splenic atrophy, reduced T cell activation and splenocyte proliferation (for review see (Offner *et al.*, 2009)). A recent study by Dziennis and co-workers observed that androgens exacerbated stroke-induced immunosuppression compared to castrated male mice (Dziennis *et al.*, 2011). This observation is consistent with previous studies in peripheral disease models which indicate that androgens can have immunosuppressive effects (Olsen & Kovacs, 1996; Palaszynski *et al.*, 2004). Similarly, it has been observed that testosterone depletion or androgen receptor antagonism prevents immunosuppression following shock or trauma (Angele *et al.*, 2000). However, direct comparisons of intact versus castrated male mice following experimental stroke have not been reported. Nonetheless, these data clearly indicate the necessity of considering the physiological effects of androgens when designing future ischemic stroke studies.

Conclusion

The research and clinical community has been aware of the fact that stroke is a sexually dimorphic disease for several years, with extensive focus on the role of estrogens in providing females with relative protection. However, less attention has been given to the role of male sex steroids, androgens, in altering the male brain's response to ischemia. Epidemiological data is emerging suggesting that levels of circulating testosterone in men is correlated with incidence of cardiovascular disease and stroke, with low testosterone levels associated with increased incidence and worse outcome. In contrast, experimental studies generally observe androgens contributing to increased injury. Importantly, the vast majority of experimental stroke studies utilize young adult animals. A few stroke studies using aging animals observed a potentially beneficial effect of androgens similar to the clinical observations. Thus, it is clear that future basic science studies would benefit from the use of aging animals and consider the effects of androgens both within and outside the central nervous system.

Acknowledgments

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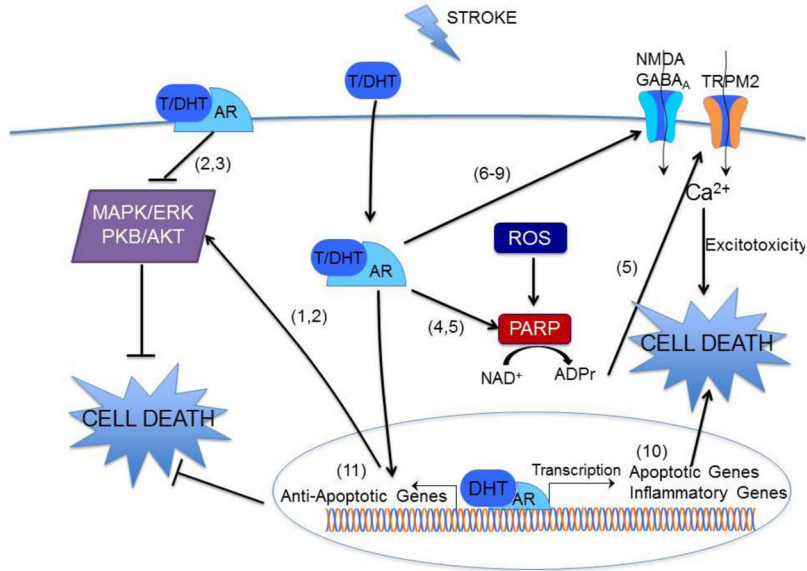
Figure 1.
Model of mechanisms of androgen neurotoxicity and neuroprotection. ADPr, adenine dinucleotide ribose; AR, androgen receptor; $GABA_A$ receptor; DHT, 5α-dihydrotestosterone; MAPK/ERK, mitogen activated protein kinases; NAD^+, nicotine adenine dinucleotide; NMDA receptor; PARP, poly(ADP ribose) polymerase; PKB/AKT, protein kinase B; ROS, reactive oxygen species; T, testosterone. Numbers in parentheses indicate source reference for each signaling pathway, (1) Nguyen *et al.* 2005 (2) Gatson *et al.,* 2006 (3) Gatson *et al.*, 2007 (4) Vagnerova *et al.*, 2010 (5) Shimizu *et al.,* 2013 (6) Caruso *et al.*, 2004 (7) Foradori *et al.*, 2007 (8) Holmes *et al.*, 2013 (9) Nunez & McCarthy, 2008 (10) Cheng *et al.,* 2007 (11) Nguyen *et al.*, 2009

Table 1
Summary of testosterone manipulation in animal models

Summary of effects of androgen manipulation in experimental stroke models.

Species/Model	Age	Manipulation	Effect on outcome	Reference
Rat/MCAO	Adult (12 weeks)	CAST; CAST+T	Cast ↓infarct; CAST+T same as intact	Hawk, 1998
Gerbil/unilateral carotid occlusion	Adult	n/a	Males incidence/injury > female	Hall, 1991
Rat/MCAO	Adult (9–10weeks)	CAST+/− T or DHT	Cast ↓infarct; CAST+T same as intact; CAST+DHT↑ infarct	Cheng, 2007
Rat/MCAO	Adult	CAST+/− T (T removed for depletion)	T depletion >6hrs protected	Yang, 2002
Rat/MCAO	Adult (8–9 weeks)	CAST+/− T replacement 1 wk post-MCAO	T accelerated recovery	Pan, 2005
Mouse/MCAO	Adult (9–10 weeks)	CAST+/− T or DHT	Low doses ↓ infarct; high doses ↑ infarct; both blocked by Flut	Uchida, 2009
Young and aged rat and mouse/MCAO	3-month-old Wistar rats and C57/BL6 mice, 14-month-old Wistar rats and 12-month-old C57/BL6 mice	CAST (in young) +/− T	T in young ↑ infarct; T in middle-aged ↓ infarct; both blocked by Flut	Cheng, 2009
Mouse/MCAO	Adult (8–10 weeks)	CAST +/− T	PARP inhibitor protection lost in CAST; restored in CAST+T	Vagnerova, 2010
Mouse/CA/CPR	Adult, 12 weeks	+/− T	Low and high doses ↑injury; intermediate had no effect	Nakano, 2010
Rat/Mouse/MCAO	Adult male Wistar rats and C57/BL6 mice (12 weeks)	CAST+/− DHT	DHT protective; increases SIK1 expression	Cheng, 2011
Mouse/MCAO	Adult (8–10 weeks)	CAST+/− DHT	DHT increased post-stroke immunosuppression	Dziennis, 2011
Mouse/MCAO	Adult (9 to 10 weeks)	CAST+/− DHT	TRPM2 inhibitor protection lost in CAST; restored in +DHT	Shimizu, 2013
Rat/Kainate	Adult (10–12 weeks)	CAST+/− DHT	CAST↑ injury; CAST+DHT same as intact	Ramsden, 2003
Rat/MCAO	Adult (12 weeks)	CAST; Flut; letrozole	CAST and Flut↓ infarct; letrozole ↑	Fanaei, 2013

Middle cerebral artery occlusion (MCAO); Cardiac arrest/cardiopulmonary resuscitation (CA/CPR); Castration/gonadectomy (CAST); Testosterone (T); Dihydrotestosterone (DHT)