

# Testosterone decreases reactive astroglia and reactive microglia after brain injury in male rats: role of its metabolites, oestradiol and dihydrotestosterone

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**Keywords:** androgen receptors, gliosis, hippocampus, major histocompatibility complex-II, stab wound injury, vimentin, Wistar rats

## Abstract

Previous studies have shown that the neuroprotective hormone, testosterone, administered immediately after neural injury, reduces reactive astrogliosis. In this study we have assessed the effect of early and late therapy with testosterone or its metabolites, oestradiol and dihydrotestosterone, on reactive astroglia and reactive microglia after a stab wound brain injury in orchidectomized Wistar rats. Animals received daily s.c. injections of testosterone, oestradiol or dihydrotestosterone on days 0–2 or on days 5–7 after injury. The number of vimentin immunoreactive astrocytes and the volume fraction of major histocompatibility complex-II (MHC-II) immunoreactive microglia were estimated in the hippocampus in the lateral border of the wound. Both early and delayed administration of testosterone or oestradiol, but not dihydrotestosterone, resulted in a significant decrease in the number of vimentin-immunoreactive astrocytes. The volume fraction of MHC-II immunoreactive microglia was significantly decreased in the animals that received testosterone or oestradiol in both early and delayed treatments and in animals that received early dihydrotestosterone administration. Thus, both early and delayed administration of testosterone reduces reactive astroglia and reactive microglia and these effects may be at least in part mediated by oestradiol, while dihydrotestosterone may mediate part of the early effects of testosterone on reactive microglia. In conclusion, testosterone controls reactive gliosis and its metabolites, oestradiol and dihydrotestosterone, may be involved in this hormonal effect. The regulation of gliosis may be part of the neuroprotective mechanism of testosterone.

## Introduction

The brain is a target for the gonadal and adrenal hormone, testosterone. Acting on the brain, the hormone regulates reproduction and sexual and aggressive behaviours (Christiansen, 2001; Wilson, 2001; Bancroft, 2005; Soma, 2006). In addition, testosterone is analgesic and anxiolytic (Edinger & Frye, 2005), affects mood and cognition (Christiansen, 2001; Cherrier, 2005; Janowsky, 2006) and promotes synaptic plasticity (MacLusky *et al.*, 2006). Testosterone also prevents neuronal death in different experimental models of neurodegeneration, and decreased testosterone levels in plasma may represent a risk factor for the development of neurodegenerative diseases in humans (Bialek *et al.*, 2004; Gold & Voskuhl, 2006; Pike *et al.*, 2006).

Control of gliosis may be one of the mechanisms involved in the neuroprotective effects of testosterone. During brain development testosterone affects the differentiation of glial fibrillary acidic protein (GFAP) immunoreactive astrocytes and generate sex differences in astroglia (Garcia-Segura *et al.*, 1988; Chowen *et al.*, 1995; Mong *et al.*, 1999; Conejo *et al.*, 2005). In the adult brain, testosterone regulates the

expression of GFAP in the hippocampus (Day *et al.*, 1993) and reduces the increase of GFAP associated to ageing in the cerebellum (Day *et al.*, 1998). In addition, testosterone reduces GFAP immunoreactivity in the border of a stab wound in the cerebral cortex and the hippocampus (Garcia-Estrada *et al.*, 1993, 1999), decreases GFAP immunostaining and astrocyte hypertrophy around the infarct area after middle cerebral artery occlusion (Pan *et al.*, 2005) and attenuates the astroglial reaction in the facial motor nucleus after facial nerve transection (Jones, K.J. *et al.*, 1997; Coers *et al.*, 2002) and in the red nucleus after rubrospinal tract transection (Storer & Jones, 2003). While these studies suggest that testosterone regulates reactive astroglia after injury, there is no available information concerning possible effects of the hormone on reactive microglia. In addition, in the previous studies that have reported an effect of testosterone administration on the number of GFAP reactive astrocytes, the hormone was administered immediately after neural injury, before the induction of reactive gliosis, and therefore it was unknown whether the hormone may affect gliosis when glial cells are already activated. Therefore, in this study we have assessed the effect on reactive astrocytes and reactive microglia of early and late testosterone therapy after a stab wound injury affecting the hippocampal formation. In addition, as testosterone metabolism within the brain may mediate many of the hormonal actions (Melcangi *et al.*, 1999; Garcia-Segura *et al.*, 2003; Balthazart *et al.*, 2004; Negri-Cesi

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Received 3 January 2007, revised 12 March 2007, accepted 27 March 2007

*et al.*, 2004; Edinger & Frye, 2005; Soma, 2006; Trainor *et al.*, 2006), we have assessed in this model the effect of two main testosterone metabolites, oestradiol and dihydrotestosterone.

## Materials and methods

### *Animals and experimental treatments*

Wistar albino male rats from the Complutense University animal colony were kept in a 12-h light : 12-h dark schedule and received food and water *ad libitum*. Animals were handled in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals, the principles presented in the Guidelines for the Use of Animals in Neuroscience Research by the Society for Neuroscience and following the European Union guidelines (Council Directive 86/609/EEC). Experimental procedures were approved by our institutional animal use and care committee, Ethical Committee for Animal Welfare at the National Paraplegics Hospital (CEBA) and Ethical Committee of CSIC. Special care was taken to reduce the number of animals used, to the minimum required for statistical accuracy. At the age of two months, animals were bilaterally orchidectomized under halothane anaesthesia to reduce circulating levels of testicular secretions. One month after orchidectomy, animals

were anaesthetized with halothane and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). An incision of the scalp was made and the cranium exposed. Then, a unilateral opening of the skull was made with a dental drill. A solid stainless cannula, 0.45 mm main diameter, was used to make a longitudinal stab wound in the left hemisphere. The cannula was positioned at 2 mm lateral to the midline and 2 mm posterior to bregma and was introduced into the brain until its tip reached a depth of 5.5 mm. Then, the cannula was displaced caudally 3 mm (bregma -5 mm) and finally removed from the brain. Bleeding was inhibited by compression with a gel-foam sponge. The scalp wound was sutured with silk.

The effects of early and late treatments after injury with testosterone or its metabolites, oestradiol and dihydrotestosterone were assessed. Therefore, a group of animals received one subcutaneous injection of testosterone (5 mg/kg), oestradiol (1 mg/kg) or dihydrotestosterone (5 mg/kg) immediately after injury, a second injection 24 h latter and a third injection 48 h latter (early steroid administration, days 0, 1 and 2 after injury). Another group of animals were injected with the same steroids at the same doses on days 5, 6 and 7 after injury (delayed steroid administration). The weight of the animals at the moment of the injections was approximately 300 g. The doses of steroids were selected on the basis of previous studies (Garcia-Estrada *et al.*, 1993) and are known to result in high physiological levels of the steroids in

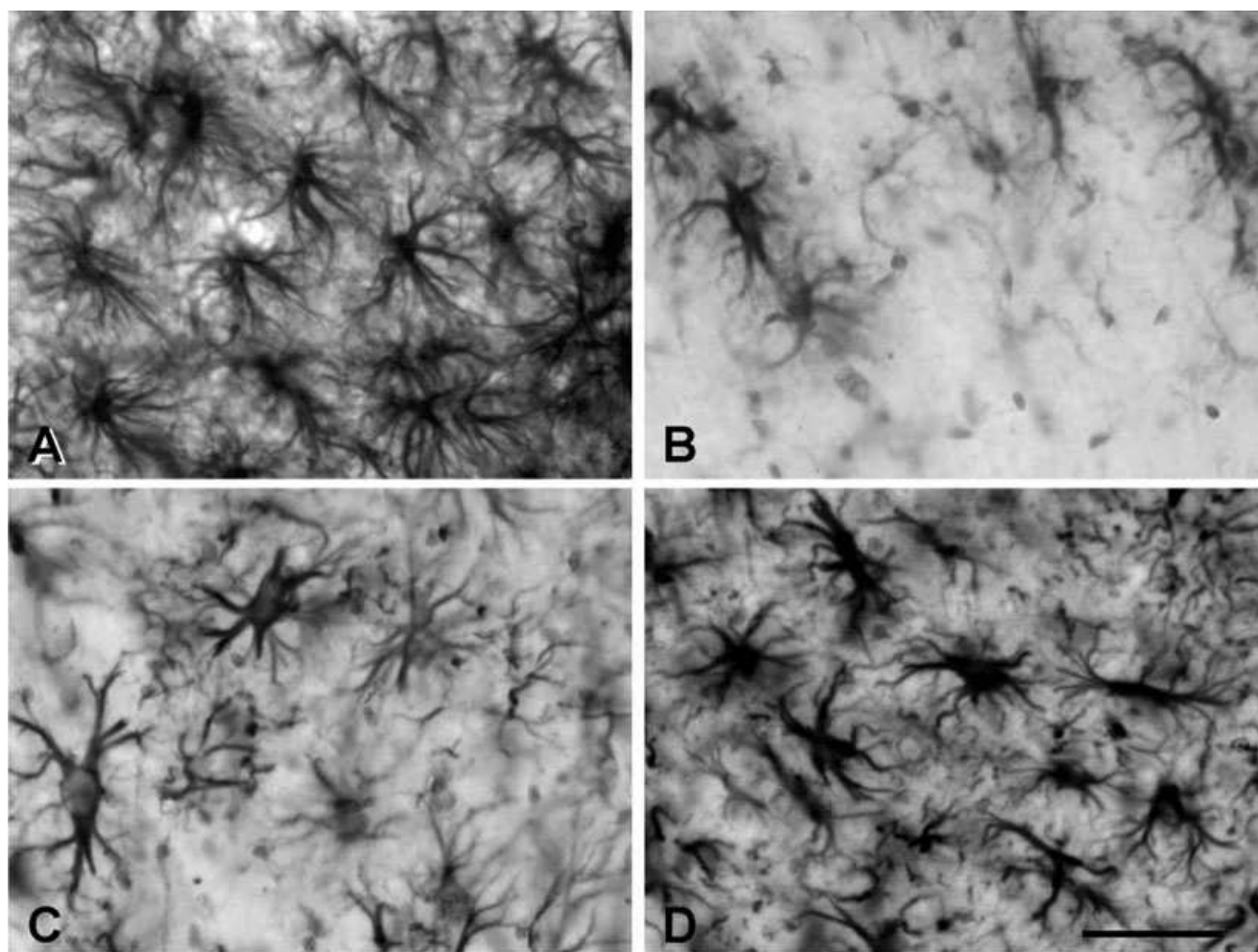


FIG. 1. Vimentin immunoreactive astrocytes in the CA1 stratum radiatum at a distance of approximately 100–200  $\mu$ m from the lateral border of the wound. The panels illustrate representative examples from orchidectomized rats after early administration of (A) vehicle; (B) testosterone; (C) oestradiol and (D) dihydrotestosterone. All figures are at the same magnification. Scale bar, 50  $\mu$ m.

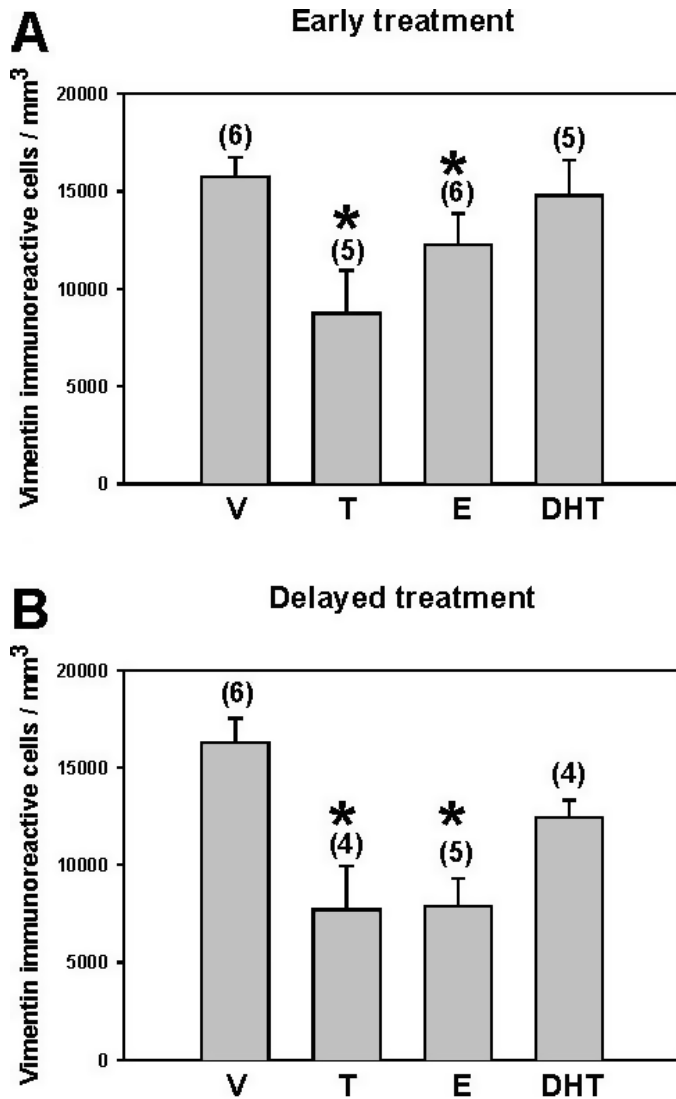


FIG. 2. Number of vimentin immunoreactive astrocytes per mm<sup>3</sup> within a distance of 350  $\mu$ m from the lateral border of the wound in animals injected with vehicle (V), testosterone (T), oestradiol (E) or dihydrotestosterone (DHT). (A) Early treatments (0–2 days after injury). (B) Delayed treatments (5–7 days after injury). Data are represented as means  $\pm$  SEM. The number of animals in each experimental group is indicated in brackets. \*Significant difference ( $P < 0.05$ ) vs. vehicle values.

plasma, to stimulate neuroprotective signalling cascades and to exert neuroprotection in the hippocampus (Lynch & Story, 2000; Cardona-Gomez *et al.*, 2002; Picazo *et al.*, 2003). In addition, androgens were injected at doses that are able to reverse the effect of castration on androgen receptor levels (Lynch & Story, 2000; Frye & Reed, 1998). Repeated injections of hormone during the three separated days were chosen to first increase the expression of androgen receptors (Frye & Reed, 1998; Lynch & Story, 2000) and then to maintain high levels of hormone around the period of glial activation (early steroid administration) or when the glial cells are already activated (delayed steroid administration).

#### Tissue fixation and immunohistochemistry

One week after brain injury, animals were deeply anaesthetized with pentobarbital (100 mg/kg, Normon Veterinary Division, Madrid,

Spain) and perfused through the left cardiac ventricle, first with 50 mL saline solution (0.9% NaCl) and then with 250 mL fixative solution (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4). Brains were removed and immersed overnight at 4 °C in the same fixative solution and then rinsed with phosphate buffer. Frontal sections of the brain, 50- $\mu$ m thick, were obtained using a Vibratome (VT 1000 S, Leica Microsystems, Wetzlar, Germany).

Immunohistochemistry was carried out in free-floating sections under moderate shaking. All washes and incubations were performed in 0.1 M phosphate buffer pH 7.4, containing 0.3% bovine serum albumin and 0.3% Triton X-100. The endogenous peroxidase activity was quenched for 10 min at room temperature in a solution of 1% hydrogen peroxide in 30% methanol. After several washes in buffer, sections were incubated overnight at 4 °C with a monoclonal antibody for vimentin (diluted 1 : 500; Clone V9, DAKO, Barcelona, Spain) or a monoclonal antibody for major histocompatibility complex-II (MHC-II; diluted 1 : 250; MCA46G, Serotec, Bicester, United Kingdom). Sections were then rinsed in buffer and incubated for 2 h at room temperature with biotinylated goat anti-mouse immunoglobulin G (diluted 1 : 300; Pierce, Rockford, IL, USA). After several washes in buffer, sections were incubated for 90 min at room temperature with avidin-biotin-peroxidase complex (diluted 1 : 250; ImmunoPure ABC peroxidase staining kit, Pierce). The reaction product was revealed by incubating the sections with 2  $\mu$ g/mL 3,3'-diaminobenzidine (Sigma-Aldrich, Tres Cantos, Spain) and 0.01% hydrogen peroxide in 0.1 M phosphate buffer. Then, sections were dehydrated, mounted on gelatinized slides, counterstained with toluidine blue and observed with a Leica DMRB-E microscope.

#### Morphometric analysis

Only brains that showed a complete lesion from the dorsal to the ventral limit of the hippocampus were selected for morphometry. The number of vimentin immunoreactive astrocytes was assessed from the dorsal to the ventral limit of the hippocampus and within a distance of 350  $\mu$ m from the lateral border of the wound. The number of vimentin immunoreactive cells was estimated by the optical disector method (Howard & Reed, 1998) using total section thickness for disector height (Hatton & von Bartheld, 1999) and a counting frame of 55  $\times$  55  $\mu$ m. A total of 78 counting frames were assessed per animal. Section thickness was measured using a digital length gauge device (Heidenhain-Metro MT 12/ND221; Traunreut, Germany) attached to the stage of a Leitz microscope. Cell nuclei from vimentin immunoreactive cells that came into focus while focusing down through the disector height were counted. All counts were performed on coded sections. The volume of the hippocampus was estimated using the point-counting method of Weibel (1979). As no significant differences in this parameter were observed among the different experimental groups, the changes in the number of vimentin immunoreactive cells per unit volume with the optical disector method are assumed to reflect changes in vimentin immunoreactive cell content.

The high density of MHC-II immunoreactive cell bodies and processes in the proximity of the wound impeded the accurate identification of individual cells. Therefore, evaluation of cell numbers using the optical disector method was not possible in this case. As an alternative, the volume fraction of MHC-II immunoreactive microglia was estimated according to the point-counting method of Weibel (1979). The outline of the hippocampus lateral to the border of the wound and the areas filled with immunoreactive material were drawn on a paper using a Leitz microscope equipped with a camera lucida. A transparent point grid was superimposed on the drawings. The total

number of points falling on the hippocampus (reference volume) and the number of points falling on immunoreactive material were counted within a distance of 350  $\mu\text{m}$  from the lateral border of the wound. The area associated to each point was 614  $\mu\text{m}^2$ . The volume fraction of immunoreactive material was calculated for each animal as the ratio of the sum of the number of points falling on immunoreactive material vs. the sum of the number of points falling in the reference volume. At least three sections were evaluated for each animal. All counts were performed on coded sections and drawings.

The  $n$  used for statistical analysis was the number of animals and is indicated in the figures. Data were analysed using Kruskal–Wallis, with  $P < 0.05$  considered to be significant.

## Results

In this study we have assessed the effect of testosterone on reactive astroglia and reactive microglia in castrated male rats. To determine whether the effects of testosterone could be mediated by its metabolites, some animals were injected with oestradiol or dihydrotestosterone. The qualitative inspection of the sections immunostained for vimentin revealed a prominent glial scar along the borders of the wound. Qualitative differences were observed between the different experimental groups. Animals treated with testosterone and

oestradiol showed a glial scar with a lower cellular density compared to control animals injected with vehicle (Fig. 1). The difference was observed both after early (days 0–2 after lesion) and delayed (days 5–7 after lesion) treatments.

The morphometric analysis with the optical disector method confirmed the qualitative observations. Both early (Fig. 2A) and delayed (Fig. 2B) administration of testosterone resulted in a significant decrease in the number of vimentin-immunoreactive astrocytes in the studied area (0–350  $\mu\text{m}$  from the lateral border of the wound). Early and delayed treatments with oestradiol also resulted in a decrease in the number of vimentin-immunoreactive astrocytes compared to control values (Fig. 2A and B). Dihydrotestosterone administration, either early or delayed, did not affect the number of vimentin immunoreactive astrocytes (Fig. 2A and B).

MHC-II immunoreactive cells were observed in all animals along the border of the lesion (Fig. 3). The volume fraction of MHC-II immunoreactive microglia showed a significant decrease in the animals that received testosterone or oestradiol in both early and delayed treatments (Fig. 4A and B). Furthermore, early administration of dihydrotestosterone significantly reduced the volume fraction of MHC-II immunoreactive cells (Fig. 4A). In contrast, the volume fraction of MHC-II immunoreactive cells was not affected by the delayed administration of dihydrotestosterone (Fig. 4B).

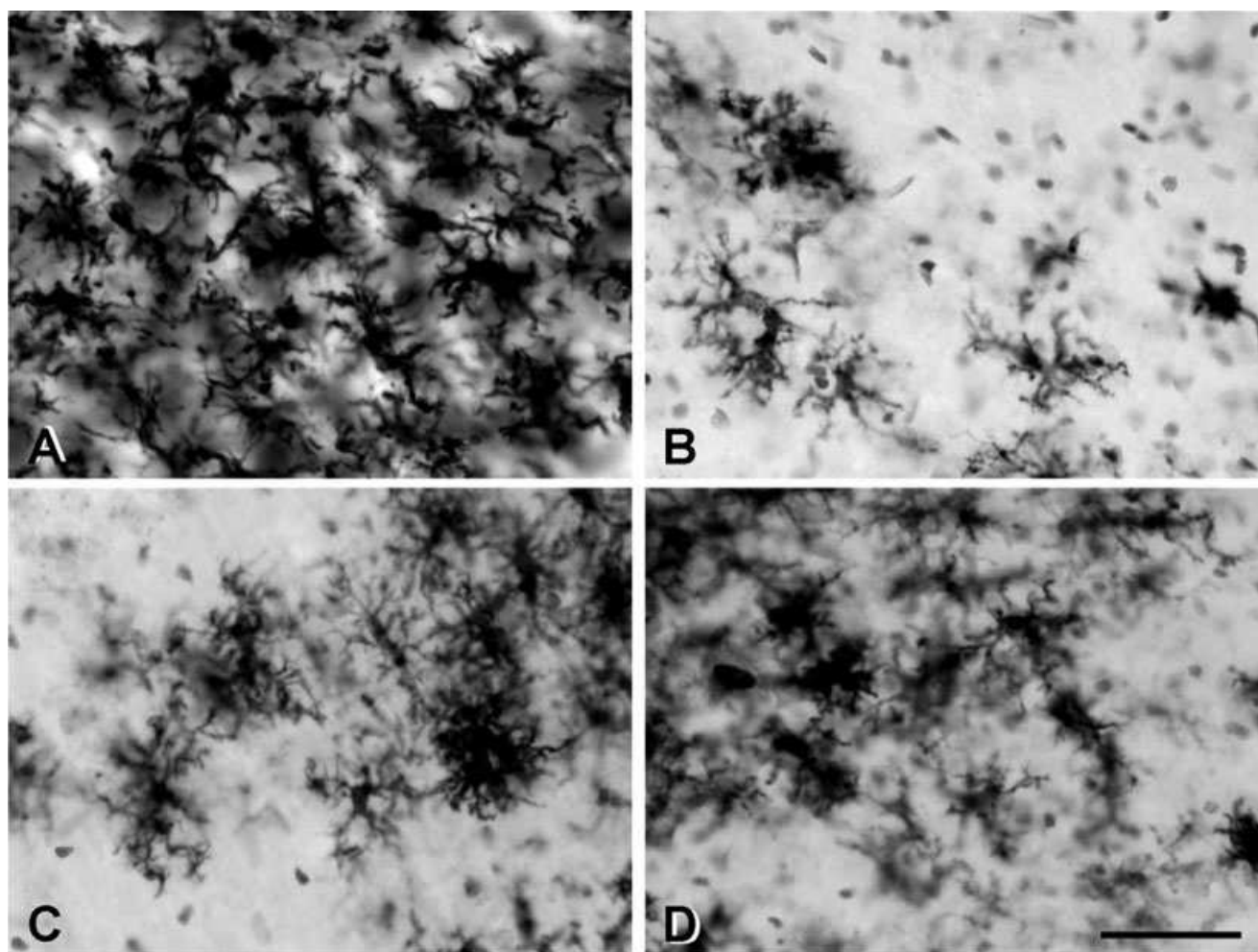


FIG. 3. MHC-II immunoreactive microglia in the CA1 stratum radiatum at a distance of approximately 100–200  $\mu\text{m}$  from the lateral border of the wound. The panels illustrate representative examples from orchidectomized rats after early administration of (A) vehicle; (B) testosterone; (C) oestradiol and (D) dihydrotestosterone. All figures are at the same magnification. Scale bar, 50  $\mu\text{m}$ .

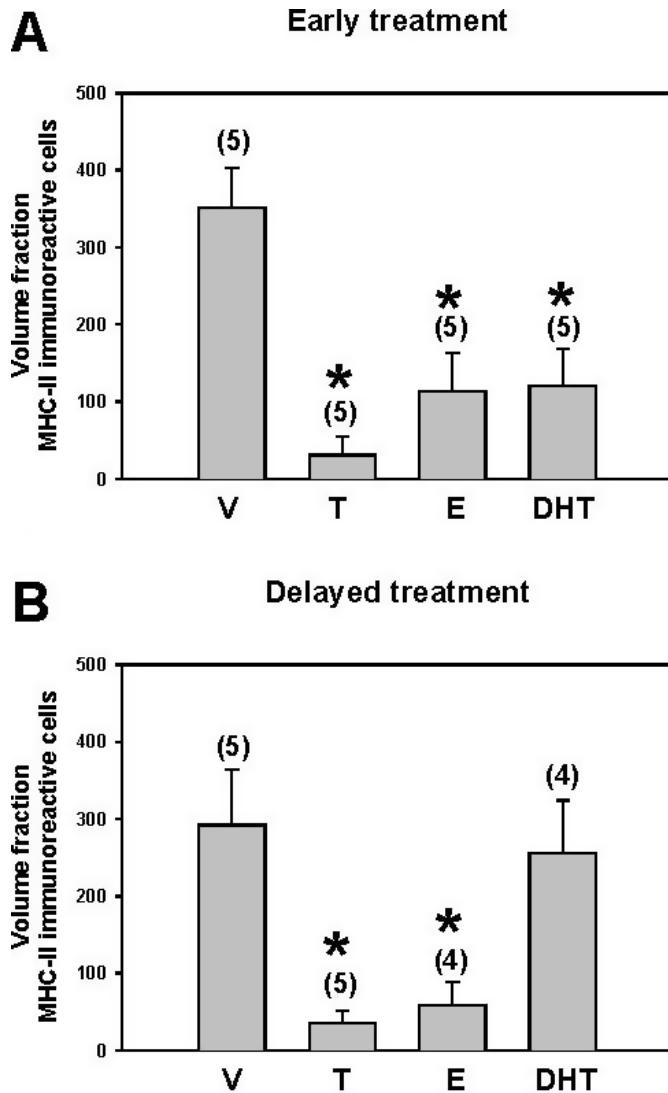


FIG. 4. Volume fraction of MHC-II immunoreactive microglia within a distance of 350  $\mu$ m from the lateral border of the wound in animals injected with vehicle (V), testosterone (T), oestradiol (E) or dihydrotestosterone (DHT). (A) Early treatments (0–2 days after injury). (B) Delayed treatments (5–7 days after injury). Data are represented as means  $\pm$  SEM. The number of animals in each experimental group is indicated in brackets. \*Significant difference ( $P < 0.05$ ) vs. vehicle values.

## Discussion

Our findings indicate that testosterone, even if administered several days after brain injury, down regulates reactive astroglia and reactive microglia. Reactive gliosis is a complex phenomenon that includes a mixture of positive and negative responses for neuronal survival and regeneration. Reactive astroglia maintains the integrity of the blood–brain barrier and the survival of perilesional tissue, but may prevent axonal regeneration (Bush *et al.*, 1999; Cui *et al.*, 2001; Faulkner *et al.*, 2004; Silver & Miller, 2004; Myer *et al.*, 2006). Reactive microglia exert important positive functions by remodelling the damaged tissue, but release pro-inflammatory cytokines and may exacerbate neuronal damage (Lindsay, 1986; Thomas, 1992; Ridet *et al.*, 1997; Streit *et al.*, 1999; Kim & de Vellis, 2005; Pekny & Nilsson, 2005). The identification of factors that regulate reactive gliosis is of practical interest for the development of therapeutic strategies to reduce neural damage and promote regeneration after

central nervous system injuries and to decrease neuronal death in neurodegenerative disorders. The activation of glial cells is modulated by local factors and by substances transported by the systemic circulation, such as the hormones secreted by the gonads and adrenals (Garcia-Segura & Melcangi, 2006).

Previous studies have shown that systemic administration of testosterone to orchidectomized male rats on days 0, 1 and 2 after a stab wound in the cortex and hippocampus, significantly decreases the number of GFAP immunoreactive astrocytes in the border of the wound (Garcia-Estrada *et al.*, 1993). In the present study we have used vimentin as a marker of reactive astrocytes (Eddleston & Mucke, 1993; Janeczko, 1993; Ridet *et al.*, 1997). Under normal conditions, only a few vimentin immunoreactive astrocytes can be detected in the hippocampus of adult animals (Ciriza *et al.*, 2004). In contrast, in injured animals, we observed a high density of vimentin immunoreactive astrocytes forming a glial scar in the proximity of the wound. Our present findings indicate that treatment of adult orchidectomized male rats with testosterone, injected on days 0–2 after a stab wound injury, reduces the number of vimentin immunoreactive astrocytes in the hippocampus in the proximity of the wound, confirming that early administration of testosterone reduces reactive astrogliosis after a stab wound in male rats (Garcia-Estrada *et al.*, 1993, 1999). In addition, one of our aims in the present study was to assess whether testosterone was also able to affect microglia activation. We have assessed microglial activation by measuring the volume fraction of MHC-II immunoreactive cells in the hippocampus in the proximity of the wound. MHC-II is a marker of reactive microglia (Streit *et al.*, 1999) and MHC-II immunoreactive cells are very scarce in the hippocampus of noninjured animals. In contrast, a high density of MHC-II immunoreactive cells was observed near the wound in the hippocampus of all injured animals. Our present findings indicate that early testosterone administration was able to reduce the volume fraction of MHC-II immunoreactive cells. Therefore, we may conclude that early administration of testosterone reduces both astroglia and microglia activation after a stab wound in the hippocampus.

Early administration of testosterone may decrease reactive gliosis by affecting the initial stages of the process of astroglia and microglia activation, which is associated to the induction of the expression of vimentin in astrocytes and MHC-II in microglia (Eddleston & Mucke, 1993; Ridet *et al.*, 1997; Streit *et al.*, 1999). However, delayed testosterone treatment, several days after injury, may represent a more relevant administration paradigm considering possible therapeutic interventions to control glial scar. Therefore, another aim of the present study was to assess whether delayed testosterone administration, when reactive glial cells are already present, may also affect reactive gliosis. Our findings indicate that testosterone is also able to reduce reactive astroglia and reactive microglia after a delayed administration, on days 5–7 after injury, when many astrocytes and microglia are already activated. This suggests that testosterone may reduce the survival of reactive glial cells or decrease the activated phenotype of astroglia and microglia. Regardless of the mechanism, the finding that the administration of testosterone several days after brain injury is still able to reduce reactive astroglia and reactive microglia, suggest that testosterone administration may represent a potential therapeutic approach to control gliosis.

Testosterone may exert its effects on reactive gliosis by acting on androgen receptors or after local conversion to oestradiol. Previous studies have shown that a stab wound and other forms of brain injury induce in reactive astrocytes the expression of aromatase, the enzyme that converts testosterone in oestradiol (Garcia-Segura *et al.*, 1999; Peterson *et al.*, 2001, 2004; Carswell *et al.*, 2005; Saldanha *et al.*, 2005). Furthermore, a stab wound injury induces the expression of

oestrogen receptors in reactive astroglia (Garcia-Ovejero *et al.*, 2002). Therefore, testosterone may be converted by aromatase-expressing reactive astrocytes in oestradiol and then oestradiol may act by a paracrine or autocrine mechanism on reactive astrocytes expressing oestrogen receptors. In addition, oestradiol may indirectly reduce reactive astrogliosis by preventing neuronal death. We detected in the present study that both early and late administration of oestradiol after brain injury reduce reactive astrogliosis in the borders of the wound. Therefore, it may be postulated that at least part of the early and late effects of testosterone on reactive astrogliosis may be mediated by its local conversion to oestradiol.

Local conversion to oestradiol may also in part mediate the effects of testosterone on microglia. Previous studies have shown that oestradiol may reduce microglia activation *in vitro* and in female rodents *in vivo* (Bruce-Keller *et al.*, 2000; Vegeto *et al.*, 2001, 2003, 2006) and our present findings indicate that early or late administration of oestradiol may also reduce microglia activation in male rats. Oestradiol may potentially act directly on microglia. Although the expression of oestrogen receptors in microglia in the rat brain *in vivo* has not been documented, oestrogen receptor  $\beta$  immunoreactivity has been detected in the hippocampus of adult macaque monkeys after ischemia (Takahashi *et al.*, 2004). Alternatively, the effect of oestradiol on microglia may be mediated by oestrogen receptor independent mechanisms or be indirect, mediated by oestrogen receptors expressed in astroglia, endothelial cells or neurons.

Testosterone may also be converted in the brain to its reduced metabolite, dihydrotestosterone, by the enzyme 5 $\alpha$ -reductase, which is also expressed in glial cells (Melcangi *et al.*, 1999; Negri-Cesi *et al.*, 2004). Dihydrotestosterone is a potent agonist of androgen receptors and many of the biological effects of testosterone are mediated by this metabolite. However, as dihydrotestosterone did not affect the number of vimentin immunoreactive astrocytes, we may conclude that the effect of testosterone on reactive astrogliosis is not mediated by the conversion in dihydrotestosterone and the subsequent activation of androgen receptors. Furthermore, androgen receptors have not been detected in reactive astrocytes in the rat brain. In contrast, early expression of androgen receptors has been detected in reactive microglia after a stab wound (Garcia-Ovejero *et al.*, 2002). Our present findings, indicating that early administration of dihydrotestosterone reduces the volume fraction of MHC-II immunoreactive cells in the hippocampus in the proximity of the wound, suggest that part of the early effect of testosterone on reactive microglia may be mediated by its conversion to dihydrotestosterone by the enzyme 5 $\alpha$ -reductase and the consecutive action on androgen receptors.

Both androgen and oestrogen receptors are involved in hormonal protective mechanisms in different experimental models of neurodegeneration (Ahlbom *et al.*, 2001; Garcia-Segura *et al.*, 2001; Hammond *et al.*, 2001; Wise, 2003; Dubal *et al.*, 2006). Therefore, the effect of testosterone and its metabolites on reactive gliosis may be in part secondary to decreased neuronal death mediated by the activation of nuclear steroid receptors. However, the activation of androgen and oestrogen receptors in the injured brain tissue may also result in the transcriptional down-regulation of several pro-inflammatory mediators that prolong the process of glial activation and maintain the expression of activated phenotype in astroglia and microglia (Garcia-Segura & Melcangi, 2006). Activation of nuclear steroid receptors might also promote the apoptotic elimination of reactive microglia, one of the mechanisms that may terminate reactive gliosis (Jones, K.J. *et al.*, 1997; White *et al.*, 1998; Dihne *et al.*, 2001).

In addition to actions on nuclear steroid receptors, the effects of testosterone and its metabolites may involve interactions with the membrane signalling of astroglia and microglia (Chaban *et al.*, 2004;

Pawlak *et al.*, 2005; Gatson & Singh, 2007; Lange *et al.*, 2007). It should be noted that in this study we have administered steroids at high doses, which are known to be able to activate several signalling kinases (Cardona-Gomez *et al.*, 2002). The activation of kinases by high doses of steroids may be independent from the modulation of transcriptional activity by steroid receptors; but may depend on the interaction of oestrogen and androgen receptors with specific signalling pathways (Nguyen *et al.*, 2005; Pawlak *et al.*, 2005; Mendez *et al.*, 2006; Gatson & Singh, 2007). Furthermore, high doses of steroids may exert antioxidant effects and inhibit lipid peroxidation reactions, conferring protection from cerebral ischemia (Simpkins *et al.*, 2005; Singh *et al.*, 2006). The antioxidant properties of oestradiol may be highly relevant to decrease gliosis by reducing oxidative stress mechanisms involved in glial activation (Wang *et al.*, 2006), including the activation of the nuclear transcription factor, NF- $\kappa$ B (Caccamo *et al.*, 2005). By the control of the intracellular localization of NF- $\kappa$ B (Dodel *et al.*, 1999; Ghisletti *et al.*, 2005), oestradiol may decrease the production of inducible NOS expression by microglia (Bruce-Keller *et al.*, 2000; Baker *et al.*, 2004). In addition, oestradiol may reduce the release of pro-inflammatory cytokines, such as interferon- $\gamma$  (IFN- $\gamma$ ) and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ; Dimayuga *et al.*, 2005) and decrease superoxide release and phagocytosis (Bruce-Keller *et al.*, 2000). Another important mechanism that may be associated to the effects of androgens and oestrogens on gliosis is the control of brain oedema by the enhancement of aquaporin-4 expression in parenchymal reactive astrocytes and perivascular glial processes (Gu *et al.*, 2003; Tomas-Camardiel *et al.*, 2005). Finally, sex steroids could be modulating glial reactivity by controlling the proliferation of astrocytes (Garcia-Estrada *et al.*, 1999), or modulating the secretion of growth factors related to the maturation of the glial scar, such as basic fibroblast growth factor (BFGF; Flores *et al.*, 1999; Moroz *et al.*, 2003) or transforming growth factor- $\beta$  (TGF- $\beta$ ; Buchanan *et al.*, 2000; Sortino *et al.*, 2004; Dhandapani *et al.*, 2005).

In summary, both early and delayed administration of testosterone reduces astroglia and microglia activation after a stab wound in the hippocampus. Furthermore, the early and late effects of oestradiol on reactive astrocytes and reactive microglia and the early effects of dihydrotestosterone on reactive microglia are compatible with a differential role of testosterone metabolites in the hormonal effects on gliosis. Finally, our results showing reduction of gliosis when testosterone or oestradiol are administered several days after brain injury open the possibility for a therapeutic use of these steroids to control gliotic tissue.

## Acknowledgements

Supported by Ministerio de Educación y Ciencia, Spain (SAF 2005-00272) and the European Union (EWA project: LSHM-CT-2005-518245).

## Abbreviations

GFAP, glial fibrillary acidic protein; MHC-II, major histocompatibility complex-II.

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