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ORIGINAL ARTICLE

Sex differences and influence of gonadal hormones on MK801-induced neuronal degeneration in the granular retrosplenial cortex of the rat

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Abstract MK801, PCP, and ketamine are non-competitive NMDA receptor–antagonists drugs that in humans produce psychomimetic effects and neurocognitive disturbances reminiscent to those of schizophrenia. The administration of these drugs in animals has been used as a pharmacological model to study the NMDA receptor hypofunction-hypothesis of schizophrenia. In animals, the biological effect of MK801 is dose-dependent. Low doses induce behavioral disturbances and higher doses, in addition, promote neurotoxicity in many brain regions, particularly the granular retrosplenial cortex (RSG). The neurotoxic effect of MK801 is sexually dimorphic, being females markedly more sensitive than males; however, the involvement of gonadal hormones is elusive. Here we show that a single intraperitoneal injection of 5 mg/kg of MK801 induced overt neurodegeneration in RSG of female rats, including abundant somatic degeneration in layer 4, and somatodendritic and terminal degeneration in layers 1, 4, and 5. MK801-degeneration in males was scarce and mainly evidenced by the presence of few argirophilic somas in layer 4. Ovariectomized rats were not significantly different than intact females, while orchiectomized rats showed robust MK801-toxicity. Testosterone and dihydrotestosterone (DHT) inhibit MK801-toxicity in orchiectomized rats. In ovariectomized rats only DHT, but not testosterone, prevented MK801-induced degeneration, while in intact females, DHT was only partially protective. Treatment of intact males with estradiol benzoate significantly enhanced MK801-toxicity. Altogether, our experiments indicate that non-aromatizable androgens protect RSG from MK801-toxicity, while estrogens counteract this protection. Thus, the balance of androgens and estrogens delineate the sexual dimorphism of the RSG to the toxic effect of MK801.

Keywords MK801 · Neurodegeneration · Granular retrosplenial cortex (RSG) · Gonadal hormones · Sexual dimorphism

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Introduction

In humans, non-competitive NMDA-antagonist drugs such as dizocilpine (MK801), phencyclidine (PCP), and ketamine produce psychomimetic effects and neurocognitive disturbances reminiscent to those of schizophrenia, suggesting that NMDA-hypofunction is implicated in the pathology (Olney et al. 1999; Jentsch and Roth 1999; Javitt 2007). Therefore, the administration of these drugs in animals has been used as a pharmacological model to study the NMDA receptor hypofunction-hypothesis of schizophrenia.

The biological effect of MK801 is dose-dependent (Olney et al. 1989; Allen and Iversen 1990; Sharp et al.

1991; Ellison 1994; Horváth et al. 1997; Matsuki et al. 2001). In rats, low to moderate doses (0.1–1.0 mg/kg) induce neurocognitive disturbances reminiscent to schizophrenia disabilities in humans (Mouri et al. 2007). At higher doses (5–10 mg/kg), in addition to behavioral alterations, MK801 induces somatodendritic neurodegeneration that becomes apparent in several brain regions and is particularly overt in the granular division of the retrosplenial cortex (RSG) (Bueno et al. 2003a; Noguchi et al. 2005). The RSG critically links information between the hippocampal formation and the neocortex contributing to spatial learning and memory (Van Groen et al. 2004). Interestingly, these functions are altered in MK801-treated animals suggesting that dysfunction of RSG is implicated in MK801-induced behavioral abnormalities (Wozniak et al. 1996; Zajaczkowski et al. 2000).

The neurotoxic mechanism for non-competitive NMDA-antagonist has not been established, but complex network disturbances have been proposed. It was postulated that blockade of NMDA-receptors on GABAergic neurons extinguishes inhibition of postsynaptic cholinergic or glutamatergic neurons causing an excessive release of excitatory neurotransmitters, enhancing excitatory transmission. This overstimulation of postsynaptic neurons, particularly those in the RSG, might explain the behavioral disturbances and the toxicity associated with the NMDA-hypofunction state (Olney and Farber 1995). The behavioral and neurotoxic effect of MK801 is also dependent on age, infant and adolescent are less sensitive than adult rats (Farber et al. 1995; Fix et al. 1995; Auer 1996; Matsuki et al. 2001; Noguchi et al. 2005), and varies with the strain, Sprague Dawley being more sensitive than Wistar rats (Bueno et al. 2003b). In addition, several studies showed that female rats are largely more sensitive to MK801 than males (Hönack and Löscher 1993; Fix et al. 1995; Auer 1996; Wozniak et al. 1998; Hur et al. 1999; Andiné et al. 1999; D'Souza et al. 2002; Bueno et al. 2003a), indicating that the neuronal response to the drug is sexually dimorphic. Curiously, the contribution of gonadal steroids to neuronal sensitivity to NMDA-antagonist drugs is still elusive. Here we show that gonadal hormones play a significant role in MK801-induced degeneration of RSG neurons. Specifically, non-aromatizable testosterone greatly protects RSG neurons from MK801-induced somatodendritic degeneration, while estrogen abrogates this protective effect.

Materials and methods

Animal care

Young adult (60–76 days) Wistar rats from the Ferreyra Institute vivarium of both sexes, weighing 190–270 g were housed with food and water ad libitum and in a 12 h light/dark cycle. All experiments conformed to named local and international guidelines on the ethical use of animals. Every effort was made to minimize discomfort of the animals and the overall number of animals used.

Hormone and MK801 treatments

At 45 days of age, male and female rats were anesthetized intraperitoneally (i.p.) with 5% chloral hydrate (0.5 ml/100 g body weight) and bilaterally orchiectomized and ovariectomized, respectively. Between 21 and 25 days post-surgery, the animals were treated subcutaneously for three consecutive days with either sesame oil alone (vehicle), 2 mg/kg testosterone propionate (TP; Organon S.A., Bs. As., Argentina), 5 mg/kg 5α-androstan-17β-ol-3-one (dihydrodestsoterone, DHT, Steraloids, Newport, RI), or 0.2 mg/kg estradiol benzoate (EB; Schering, Argentina). For some experiments, intact young adult rats (60–76 days) of both sexes were used with or without hormonal treatment. One day after the last hormone or vehicle injection, the animals received a single i.p. injection of NaCl (vehicle) or 5 mg/kg of dizocilpine hydrogen maleate, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK801; RBI, Natick, MA). Three days after the MK801 treatment, the animals (66–76 days of age) were sacrificed and neurodegeneration was analyzed.

Perfusion and histological procedure

At the time of sacrifice, rats were anesthetized i.p. with 30% chloral hydrate and were perfused transcardially and fixed with 4% paraformaldehyde in 0.2 M borate buffer (pH 7.4), and the brains were placed in 30% sucrose. Serial sagital sections of 40 µm were obtained in a freezing microtome and stored at 4°C until processing. Tissue sections were stained using the A–Cu–Ag technique (de Olmos et al. 1994) to visualize somatodendritic degeneration.

Amino–cupric–silver technique (A–Cu–Ag)

The A–Cu–Ag is a more recent version of the cupric–silver method and suitable for staining degenerating perikarya, dendrites, stem axons, and their terminal ramifications in brain tissue subjected to different experimental and neuropathological conditions (de Olmos et al. 1981). The procedure was carried out following the protocol previously described (de Olmos et al. 1994). Briefly, sections were rinsed in double-distilled water and incubated in a pre-impregnating solution of silver nitrate at 50°C. After cooling to room temperature, sections were rinsed with acetone and transferred to a concentrated impregnating

silver nitrate solution for 40 min. Sections were then immersed in a reducing solution for 25 min and the reaction was stopped in 0.5% acetic acid. Bleaching was done in two steps to eliminate the non-specific deposits of silver on the tissue. After stabilization in a fixer solution, the sections were mounted.

Image analysis and counting procedure

Neuronal degeneration was analyzed within layer IV of the granular RSG. A previous study (Auer 1996) showed that neuropathological changes in the RSG induced by MK801 increases caudally with an approximate peak between −5.3 and −7.3 mm with respect to bregma. Therefore, serial sagital sections were considered optimum for revealing the extent of degeneration induced by MK801 within the RSG. Argyrophilic somas revealed by the A–Cu–Ag technique were assessed at 50× magnification. At this magnification, intense silver stain of the cell body and processes of degenerating neurons were easily identified and counted by a treatment-blind investigator. The mean number of degenerating neurons in the RSG of each animal was assessed by counting the number of argyrophilic neurons in both hemispheres from three sections corresponding to lateral planes 0.9, 1.1, and 1.4 mm according to the atlas of Paxinos and Watson (2007). The mean number of degenerating neurons per animal was used for statistical analysis.

Statistics

Because animals treated with saline showed no degeneration, all statistical comparison were made using MK801-treated animals exclusively. For statistical comparisons, average number of degenerating neurons per animal was used and compared using either one- or two-way ANOVA followed by Newman–Keuls post hoc test.

Results

To address the influence of gonadal hormones in MK801-induced neurotoxicity, we used intact as well as orchiectomized and ovariectomized rats treated with a single i.p. injection of saline (control) or 5 mg/kg of MK801. All animals that received MK801 showed severe incoordination, increased motor activity, ataxia, head-weaving movements and, minutes later, recumbency. All animals were awake and moving between 6 (males) and 24 h (females) after MK801 injection. Three days after treatment animals were sacrificed and neurodegeneration was evaluated in the RSG using the A–Cu–Ag technique (de Olmos et al. 1994). Regardless of sex, saline-treated animals (both intact, orchiectomized, and ovariectomized) were free of signs of neurodegeneration neither in the RSG nor in any other brain area (data not shown, see Bueno et al. 2003a) and therefore were not included in further comparisons. On the contrary to control animals, MK801-treated intact females showed a robust argirophilic reaction depicting degenerating soma, axons, and dendrites. Degenerative profiles were not evenly distributed in the RSG, instead a gradient of increasing neurodegeneration was evident from the rostral to the caudal end (1.8–7.3 from Bregma) (Fig. 1a) and from the lateral to the middle side (0.4–1.4 mm; data not shown) of the RSG. Argirophilic somas were exclusively found in layer 4, while dendrites, axons, and terminals were observed in layers 1, 4, and 5 (Fig. 1b). Layers 2, 3, although practically free of argirophilic degeneration, were sporadically traversed by continuous or beaded fibers oriented vertically to the basal surface of the RSG. These fibers reached layer 1, where they appeared to contribute to the formation of dome-like accumulations of degenerative dendrites (thicker fragments) that intercalated with less obvious axon-like degeneration (smaller puncta) (Fig. 1a, b). There was an apparent correlation between the number of argirophilic somas in layer 4 and the density of degenerating dendrites and terminal axons in the corresponding segments of layers 1, 4, and 5 (Fig. 1b), suggesting that these structures belong to the same neurons.

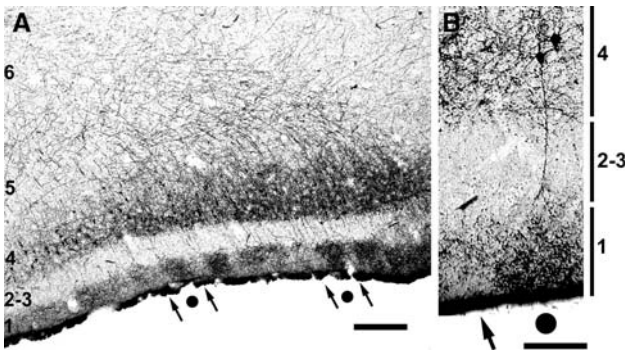

Fig. 1 MK801-induced neurodegeneration in the RSG. **a** Low-power photomicrograph of neuronal degeneration evidenced by the A–Cu–Ag technique. Shown is a section of the RSG (lat 1.4 mm) of an intact female rat treated with a single i.p. injection of 5 mg/kg of MK801 and analyzed 3 days post-treatment. Note the rostro-caudal gradient of increasing degeneration. Numbers indicate the cortical layers, *dots* point to the "dome-like" accumulation of argirophilic-dense dendritic-like granule that intercalate with axon-like argirophilic-thin puncta in layer 1 (*arrow*) (*scale bar* 100 μm). **b** Detail of neurodegenerative profiles in RSG. Shown is a high-power photomicrograph of RSG (lat 1.4 mm). Note that layers 2, 3 are practically free of degeneration except for crossing fibers emerging form neurons located in layer 4. Layer 4 shows argirophilic granules corresponding to terminal and dendritic degeneration, and neurons depicting somatodendritic degeneration. Layer 1 shows "dome-like" accumulation (*dot*) intercalated with axon-like appearance thinner granules (*arrow*). Numbers at *right* denote cortical layers (*scale bar* 50 μm)

Notably, in contrast to females, overall neuronal degeneration in MK801-treated intact males was negligible, although some degree of neurodegeneration occurred in the RSG (Fig. 2a, b). Argirophilic somas were rare and randomly distributed in layer 4 (Fig. 2b, arrowhead, and insert), but were more frequently found in the caudal end of the RSG suggesting that in males there is also a rostro-caudal gradient of neurodegeneration. Interestingly, unlike females, in males very few degenerating somas were systematically found in layer 5 of RSG (Fig. 2, arrow and insert). Dome-like structures were not evident and scarce dendritc and axonal degeneration in layer 1 do not appear to correlate with argirophilic somas in layer 4 (Fig. 2b).

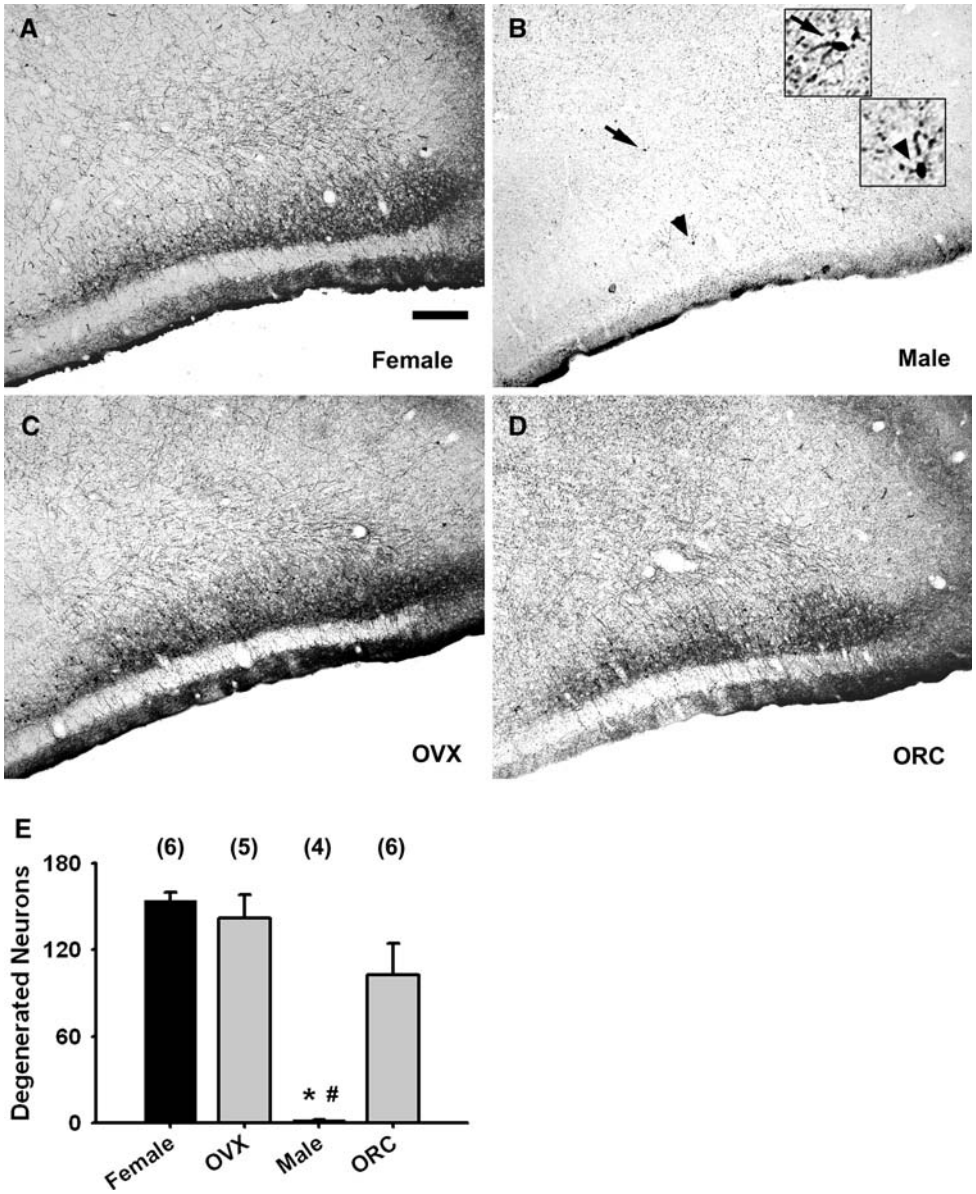

Fig. 2 Orchiectomy abolishes the sexually dimorphic effect of MK801-induced toxicity in the RSG. Intact and gonadectimized rats of both sexes were treated i.p. with 5 mg/kg of MK801, neurodegeneration in the RSG was analyzed 3 days later using the amino–cupric–silver technique. Shown are representative images of low-power photomicrographs of RSG (lat 1.4 mm) of intact female (**a**), intact male (**b**), OVX (**c**), and ORC (**d**) rats. Inserts in **b** are enlargements of argirophilic neurons in layer 4 (*arrowhead*) and layer 5 (*arrow*). Note that orchiectomy dramatically enhances MK801-toxicity of male rats. **e** Quantitative analysis of MK801-induced neurodegeneration in the RSG. The number of argirophilic somas in layer 4 of RSG (lat 0.4–1.4 mm) were scored and statistically analyzed. MK801 induced neurodegeneration in the RSG indicated a significant effect for sex ($F_{[1,17]} = 35.91$; $P < 0.000015$) and castration $F_{[1,17]} = 6.82$; $P < 0.01$) with a significant interaction sex × castration ($F_{[1,17]} = 13.03$; $P < 0.002$) by two-way ANOVA. Males were significantly different from the other groups, post hoc Neuman–Keuls test ($^{*}P < 0.0001$ vs. female and OVX, $^{\#}P < 0.0005$ vs. ORC). All other relevant comparisons were not significant. Numbers in parenthesis indicate number of cases (*scale bar* 200 μm)

Surprisingly, the effect of castration was completely different in males and females. Compared to intact females, ovariectomy did not induce a detectable change in the pattern of MK801-induced argirophilia (Fig. 1a, c). On the contrary, orchidectomized rats displayed a robust increase in neuronal degeneration that becomes similar to that of female animals including abundant argirophilic somas in layer 4 and absence of degenerating somas in layer 5 (Fig. 2a–d). In order to get a quantitative assessment of MK801-neurotoxicity, we scored the number of argirophilic somas in layer 4 of RSG. The number of argirophilic somas was similar in intact females and ovariectomized rats (OVX) (148 ± 8 and 133 ± 14, respectively). In intact males, a mean of 2 ± 0.5 somas were found and orchiectomy (ORC) significantly increased argirophilic somas to 96 ± 23. The two-way ANOVA (sex × castration) on MK801 induced neurodegeneration in the RSG indicated a significant effect for sex ($F_{[1,17]} = 35.91$; $P < 0.000015$), as well as castration ($F_{[1,17]} = 6.82$; $P < 0.01$), with a significant interaction between sex and castration ($F_{[1,17]} = 13.03$; $P < 0.002$). The following post hoc comparison using Neuman–Keuls test demonstrated that males were significantly different from the other groups ($^{*}P < 0.0001$ vs. female and OVX; $^{\#}P < 0.0005$ vs. ORC) (Fig. 2e). Altogether, these results indicate that sensitivity of RSG neurons to MK801 toxicity is sexually dimorphic and suggest that male hormones play a protective role.

To analyze the potential neuroprotective role of gonadal hormones in MK801-toxicity, ORC rats were treated with testosterone propionate (TP), dihydrotestosterone (DHT) and estradiol benzoate (EB) or the corresponding vehicle-control (vh), and toxicity of MK801 in RSG was analyzed. As expected, MK801 induced robust argirophilia in ORC + vh rats (Fig. 3a). MK801-degeneration was greatly reduced in ORC + TP (Fig. 3c). In these animals, terminal degeneration and dome-like accumulations in layer 1 were largely absent, while few scattered argirophilic somas were present in layers 4 and 5. Interestingly, DHT-treatment almost completely abolished all type of degenerating profiles including the argirophilic somas of layers 4 and 5 (Fig. 3d). MK801-degeneration in ORC rats treated with EB did not differ from that of control ORC + vh rats (Fig. 3b). Quantitative analysis of argirophilic somas in layer 4 confirms that EB did not promote a significant change while both, TP and to a greater extent DHT, significantly reduced the number for degenerating somas (Fig. 3e) (by one-way ANOVA, $F_{[3,13]} = 5.28$, $P < 0.01$). These data indicate that EB does not prevent MK801-toxicity, while androgens have a major protective role.

To further test the neuroprotective role of gonadal hormones in MK801-toxicity, the effect of vh, TP, DHT, and EB were also analyzed in OVX rats. Compared to OVX + vh rats, EB appears to have no appreciable effect on MK801-toxicity (Fig. 4a, b), consistent with the effect observed in ORC animals. Unexpectedly, TP did not prevent MK801-toxicity in OVX animals, while DHT showed a major protective effect (Fig. 4c, d). Although the protective effect of DHT in OVX rats was largely evident, scattered argirophilic somas were found in layer 4, and notably also in layer 5, which is a pattern similar to that of intact males. Terminal and dome-like accumulations were observed with a clear rostro-caudal gradient (Fig. 4d). Quantitative analysis of argirophilic somas in layer 4 confirms the significant protective effect of DHT in OVX rats (32 ± 8 somas) (by one-way ANOVA, $F_{[3,13]} = 13.97$, $P < 0.0002$), indicating that DHT also protects female RSG neurons from MK801-toxicity. Moreover, DHT, but not TP nor EB, significantly reduces MK801-toxicity in intact females (Fig. 4f) (by one-way ANOVA, $F_{[3,14]} = 3.43$, $P < 0.04$). Altogether these observations further confirm the protective effect of DHT on MK801-toxicity, and they also uncover the possibility that EB, that is present in intact females or is raised from aromatization of TP, might counteract DHT-protection. To test this possibility, intact males were treated with vh, EB, TP, and DHT and MK801-toxicity was assessed. Importantly, EB-treated males displayed a robust increase in MK801-degeneration, including the rostro-caudal gradient of degeneration, with argirophilic somas in layer 4 and terminal degeneration and dome-like accumulation in layer 1 (Fig. 5a, b), all of which is largely similar to the pattern of MK801-degeneration found in females. Quantitative analysis of argirophilic somas in layer 4 (Fig. 5c) confirms the significant enhancement of toxicity due to EB treatment (40 ± 14 somas) (by one-way ANOVA, $F_{[3,13]} = 7.6$, $P < 0.003$). Collectively, these experiments show that non-aromatizable TP protects RSG neurons to MK801-toxicity, and EB counteracts this protective effect.

Discussion

The present study shows that androgens markedly reduce the neurotoxic effect of MK801 on the RSG, while estrogens appear to counteract this protective effect. The sexual differences in the neurotoxic effect of NMDA-antagonist drugs such as MK801 and PCP have been largely recognized (Olney et al. 1989; Fix et al. 1995; Auer 1996; Wozniak et al. 1998) but the participation of gonadal hormones has not been well defined. Here we show that males are significantly less sensitive than females to MK801-toxicity and orchiectomy enhances toxicity while ovariectomy does not have a detectable effect. That is, abolishment of circulating gonadal hormones by castration abrogates the sexually dimorphic response to MK801-toxicity and degeneration in both, ORC and OVX rats,

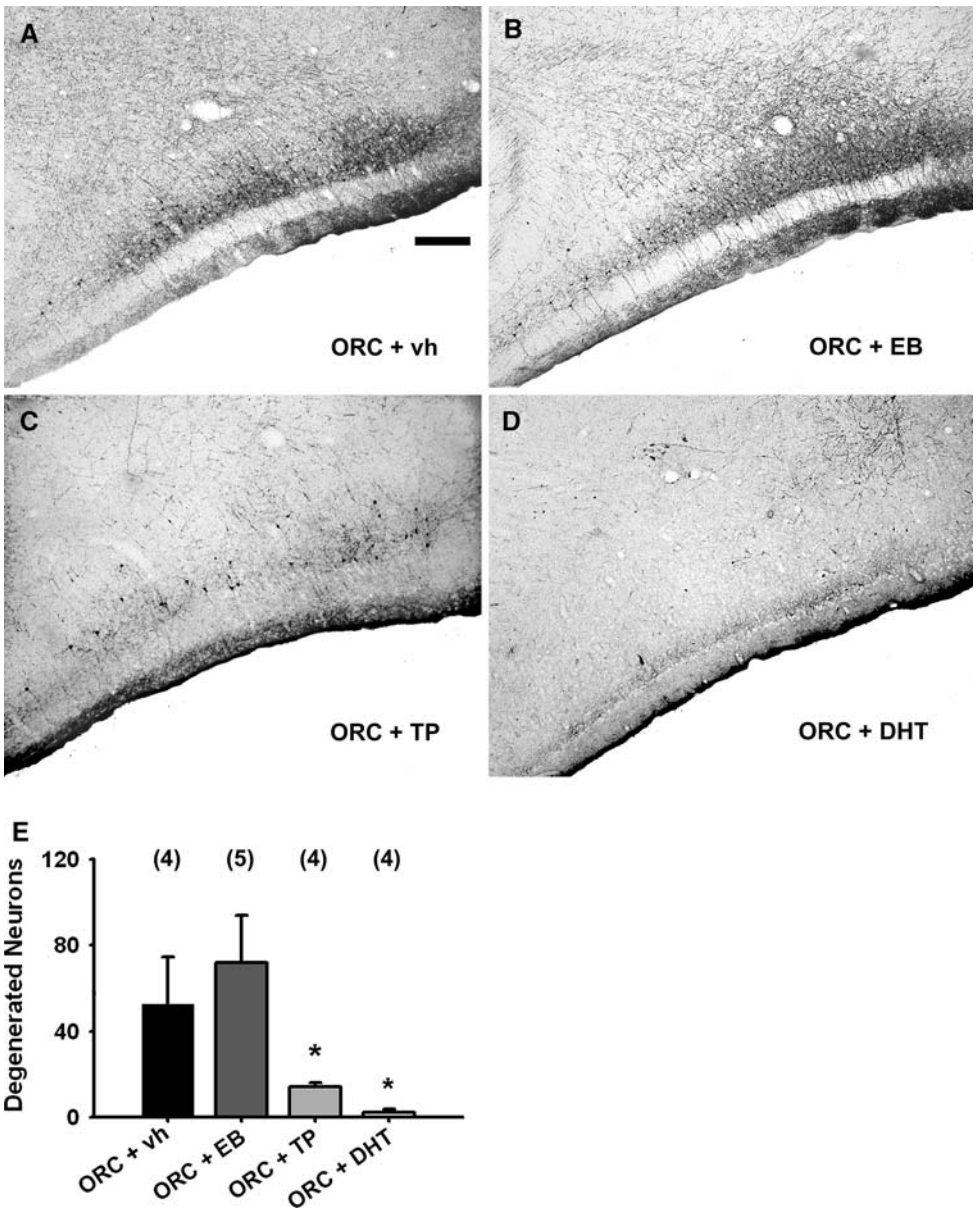

Fig. 3 Androgens inhibit MK801-induced toxicity in the RSG of ORC rats. ORC rats were treated with vh, EB, TP or DHT for three consecutive days prior to a single i.p. with 5 mg/kg of MK801 and neurodegeneration in the RSG was analyzed 3 days later using the A–Cu–Ag technique. Shown are representative images of low-power photomicrographs of RSG (lat 1.4 mm) of ORC rats treated with vh (**a**), EB (**b**), TP (**c**), and DHT (**d**). Note that TP and DHT inhibit MK801-toxicity. **e** Quantitative analysis of MK801-induced neurodegeneration in the RSG. The number of argirophilic somas in layer 4 of RSG (lat 0.4–1.4 mm) were scored and statistically analyzed (*$P < 0.03$ vs. ORC + vh by ANOVA $F_{[3,13]} = 5.28$, followed by Newman–Keuls post hoc test). In parenthesis are indicate the number of cases (*scale bar* 200 μm)

becomes indistinguishable to that of intact females. These observations strongly suggest that RSG neurons are intrinsically sensitive to MK801-toxicity, and the presence of androgens exert a protective action. Supporting this interpretation, both TP and DHT replacement significantly reduced MK801-toxicity in ORC animals. Moreover, DHT also reduced toxicity of MK801 in OVX animals. Interestingly, on the contrary to DHT, TP was not protective in OVX animals, suggesting that its aromatization to estrogens caused the loss of its neuroprotective effect. This observation suggests that estrogens can oppose or counteract the protective effect of TP. Several lines of evidence suggest that estrogen per se does not sensitize RSG neurons to MK801-toxicity, but rather abolishes the neuroprotective effect of TP. First, EB treatment does not enhance MK801-toxicity in neither ORC, OVX nor in intact female rats. Second, ovariectomy does not reduce MK801-toxicity, indicating that gonadal estrogens are not required to induce or enhance MK801-toxicity. Third, the neuroprotective effect of DHT is much less effective in intact females (with endogenous gonadal estrogens) than in OVX rats (with no gonadal estrogens), and fourth, our data and those presented by Hur et al. (1999) show that EB treatment significantly enhances MK801-toxicity in intact male with endogenous levels of gonadal androgens. Taken together, these observations argue for a neuroprotective role of DHT in MK801-toxicity, while EB counteracts this effect. Thus, the relative balance between androgens and estrogens may delineate the sexually dimorphic response of RSG neurons to the degenerative effect of MK801.

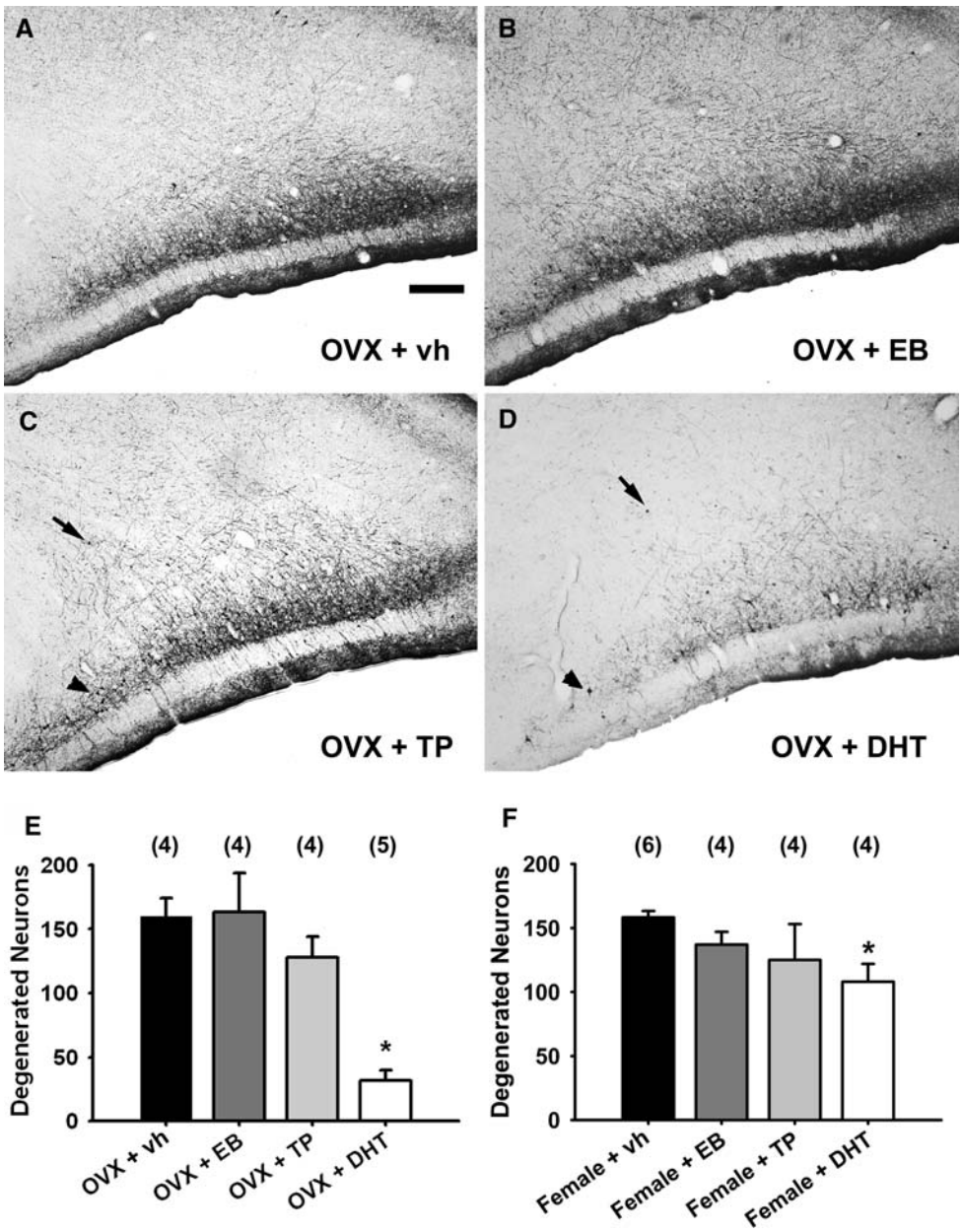

Fig. 4 Dihydrotestosterone, but not testosterone, inhibits MK801-induced toxicity in the RSG of OVX and intact female rats. OVX rats were treated with vh, EB, TP, or DHT for three consecutive days prior to a single i.p. with 5 mg/kg of MK801 and neurodegeneration in the RSG was analyzed 3 days later using the A–Cu–Ag technique. Shown are representative images of low-power photomicrographs of RSG (lat 1.4 mm) of OVX rats treated with vh (**a**), EB (**b**), TP (**c**), and DHT (**d**). Examples of degenerating neurons in layer 4 (*arrowhead*) and layer 5 (*arrow*) are shown in **c** and **d**. Note that DHT, but not TP, inhibits MK801-toxicity in OVX rats. **e**, **f** Quantitative analysis of MK801-induced neurodegeneration in the RSG of OVX rats. The number of argirophilic somas in layer 4 of RSG (lat 0.9–1.4 mm) were scored and statistically analyzed. **e** $^{*}P < 0.0007$ versus OVX + vh by ANOVA, $F_{[3,13]} = 13.97$ followed by Newman–Keuls post hoc test. **f** $^{*}P < 0.04$ versus female + vh by ANOVA, $F_{[3,14]} = 3.43$ followed by Newman–Keuls post hoc test. All other relevant comparisons were not significant. In parenthesis are indicate the number of cases (*scale bar* 200 μm)

The mechanisms by which gonadal hormones triggers the sexually dimorphic response to MK801-induced neuronal degeneration in RSG remain unclear, and both, peripheral and central effects may be involved. It has been previously shown that the half-life of MK801 in plasma and brain is much longer in females than in males (Andiné et al. 1999). Interestingly, the hepatic catabolism of PCP, another non-competitive NMDA-antagonist drug, was found to be sexually dimorphic resulting in a much longer half-life in the female (Nabeshima et al. 1984a, b; Shelnutt et al. 1999). Thus, it is possible that estrogens may decrease the metabolic activity of the liver altering the pharmacokinetics of MK801 and thereby increasing its half-life and availability to blockade of NMDA-receptors in the brain. Although the peripheral effect of gonadal hormones cannot be completely ruled out, some of our observations strongly support a direct action of gonadal steroids in the CNS as the main mechanism implicated in the sexually dimorphic response to MK801-toxicity. In intact males, but not in intact females, we systematically found scattered argirophilic neurons in layer 5 of the RSG after MK801-treatment. Interestingly, orchiectomy precludes argirophilic staining of these neurons and both, TP and DHT, restores degenerating profiles in layer 5, indicating that androgens sensitizes those neurons to the toxic effect of the drug. Notably, treatment of OVX rats with DHT is sufficient to induce MK801-neurodegeneration in layer 5 neurons, further confirming that non-aromatizable

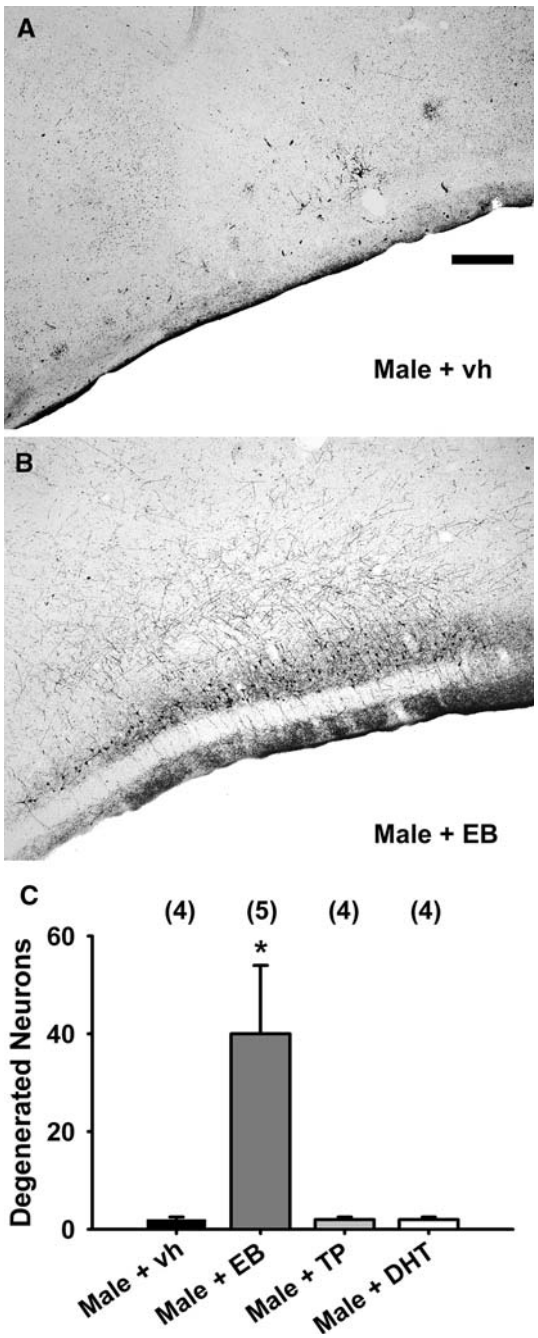

Fig. 5 Estradiol enhances MK801-induced toxicity in the RSG of intact male rats. Intact male rats were treated with vh, EB, TP, or DHT for three consecutive days prior to a single i.p. of 5 mg/kg of MK801 and neurodegeneration in the RSG was analyzed 3 days later using the A–Cu–Ag technique. Shown are representative images of low-power photomicrographs of RSG (lat 1.4 mm) of male rats treated with vh (**a**) and EB (**b**). Note that EB enhances MK801-toxicity in the RSG. **c** Quantitative analysis of MK801-induced neurodegeneration in the RSG of male rats. The number of argirophilic somas in layer 4 of RSG (lat 0.4–1.4 mm) were scored and statistically analyzed. $^*P < 0.003$ versus male + vh by ANOVA, $F_{[3,13]} = 7.6$ followed by Newman–Keuls post hoc test. All other relevant comparisons were not significant. In parenthesis are indicate the number of cases (*scale bar* 200 μm)

androgens render these neurons sensible to MK801-toxicity. Although the physiopathological significance of the degenerating neurons in layer 5 is elusive, they strongly argue for a central rather than peripheral effect of gonadal hormones on the sexually dimorphic effect of MK801.

The central effect of gonadal hormones may be exerted through a plethora of different ways, including modulating gene expression, activating signaling cascades, maintaining Ca^{2+} homeostasis, modulating neurotrophins action, among many others. For example, gonadal hormones may modulate MK801-toxicity by altering the expression and/or availability of receptors for neurotransmitters. It was found that orchiectomy decreases binding of MK801 in subfields of the septum and hypothalamus (Kus et al. 1995a) and augments binding of MK801 in hippocampal CA1 region (Kus et al. 1995a, b), an area that expresses relatively high levels of androgen receptors (Simerly et al. 1990; Handa et al. 1987). Importantly, these alterations can be reversed by DHT-treatment (Kus et al. 1995a, b), indicating that binding of MK801 in some brain areas can be modulated by circulating androgens. In addition, androgens can alter the excitability of CA1 pyramidal neurons (Pouliot et al. 1996; Smith et al. 2002), and protects them against NMDA-induced excitotoxicity (Pouliot et al. 1996). Thus, it is possible that circulating androgens may modulate NMDA-receptor function and thereby prevent MK801-induced neuronal degeneration.

Curiously, our experiments suggest that the protective effect of androgens is counteracted by estrogen. This action of estrogens is unexpected since, through their alpha-receptor, are known to be protective for cortical and hippocampal neurons from different injuries (Shughrue and Merchenthaler 2003; Dubal et al. 2001). Dribben et al. (2003) showed that estradiol promotes a reduction in MK801-induced vacuolated neurons, suggesting a potential neuroprotective action of estrogens in mild reversible neurotoxicity of MK801. However, our results strongly indicated that estrogen is not protective to MK801-irreversible neurotoxicity, and rather oppose to the protective effect of testosterone. It is unclear whether estrogens and androgens act opposite on the same molecular target or have activities on distinct pathways. Several lines of evidence indicate that estradiol enhances hippocampal NMDA receptor-mediated responses, increases NMDA-receptor binding, and expression of the NR1 subunit of the NMDA-receptor in CA1 (Gazzaley et al. 1996; Weiland 1992; Wong and Moss 1992, 1994; Woolley et al. 1997; Cyr et al. 2000, 2001). Estradiol increases neuronal excitability and the repetitive firing (Wong and Moss 1992) reducing the threshold for seizure (Hom and Buterbaugh 1986; Buterbaugh 1989; Woolley 2000). In addition, EB could also influence MK801-induced neurotoxicity by decreasing

GABAergic function (Murphy et al. 1998; Rudick and Woolley 2001) and thereby decreasing tonic levels of inhibition. Interestingly, GABAergic agonists such as benzodiazepines and barbiturates prevent neurotoxicity in RSG induced by MK801 or PCP treatment (Olney et al. 1991). Thus, estrogens and androgens may be able to modulate the dimorphic response to MK801 by acting on the same target such as glutamatergic transmission or by a complex modulation of a polysynaptic circuit involving several neurotransmitters.

The onset of symptoms of schizophrenia that normally occurs during adolescence suggests a relationship between this disorder and gonadal hormones. Non-competitive NMDA-antagonist drugs such as MK801 and PCP have been proposed as a model of schizophrenia because these drugs induce schizophrenic symptoms in humans (for review see Mouri et al. 2007). Recent studies have shown that levels of circulating androgens inversely correlate with the severity of negative symptoms in male patients with schizophrenia (Akhondzadeh et al. 2006; Ko et al. 2007). Therefore it is tempting to speculate that the model reported here may be helpful for the elucidation of molecular mechanisms by which gonadal steroids might modulate behavioral dysfunction induced by NMDA-hypofunction in some psychotic syndromes.

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