NANOMOLAR CONCENTRATIONS OF ANABOLIC-ANDROGENIC STEROIDS AMPLIFY EXCITOTOXIC NEURONAL DEATH IN MIXED MOUSE CORTICAL CULTURES

Rosamaria Orlando[1*], Alessandra Caruso[1*], Gemma Molinaro[2], Marta Motolese[1], Francesco Matrisciano [1], Giuseppina Togna[1], Daniela Melchiorri[1], Ferdinando Nicoletti[1,2] and Valeria Bruno[1,2]

[1]Istituto Neurologico Mediterraneo Neuromed, Pozzilli, Italy; [2]Department of Human Physiology and Pharmacology, University of Rome "La Sapienza", Rome, Italy

The use of anabolic-androgenic steroids (AASs) in the world of sport has raised a major concern for the serious, sometimes life-threatening, side effects associated with these drugs. Most of CNS effects are of psychiatric origin, and whether or not AASs are toxic to neurons is unknown, as yet. We compared the effect of testosterone with that of the AASs, 19-nortestosterone (nandrolone), stanozolol, and gestrinone, on excitotoxic neuronal death induced by NMDA in primary cultures of mouse cortical cells. Cultures were challenged with NMDA for 10 min and excitotoxic neuronal death was assessed 18-20 hrs later. Using this paradigm of "fast toxicity", we examined the effect of a 4-day pretreatment with testosterone, 17β-estradiol, progesterone, and three AASs. Pretreatment with testosterone slightly reduced NMDA toxicity at concentrations of 10 nM, was ineffective at 100 nM – 1 μM, but amplified toxicity at 10 μM. In contrast, all three AASs enhanced NMDA toxicity at low concentrations. A 4-day pretreatment with low concentrations (10 nM) of 17β-estradiol was substantially neuroprotective against NMDA toxicity. Progesterone was protective at all concentrations tested. Because aromatase is expressed in neurons, a potential toxicity of low concentrations of testosterone could have been counterbalanced by the formation of 17β-estradiol during the 4 days of incubation in culture. To examine this possibility, testosterone was combined with aminoglutethimide (10 μM). Under this condition, testosterone was no longer neuroprotective at 10 nM, and became neurotoxic at 1 μM. Amplification of NMDA toxicity by 10 μM testosterone was abrogated by the androgen receptor antagonist, flutamide (10 μM). Amplification of NMDA toxicity by nortestosterone, stanozolol, or gestrinone was prevented by flutamide, but was insensitive to aminoglutethimide. Finally, we examined the effect of testosterone and the three AASs applied in combination with NMDA during the excitotoxic pulse. Using this particular protocol of drug treatment, testosterone did not affect NMDA toxicity, whereas nortestosterone amplified excitotoxic death at concentrations $\geq$ 100 nM, and its action was prevented by flutamide. Stanozolol amplidied NMDA toxicity only at 10 μM, whereas gestrinone was toxic at 10 and 100 nM and inactive at 10 μM. When combined with NMDA, 17β-estradiol was protective at 10 nM but not at higher concentrations, whereas progesterone was protective at all concentrations tested. These data suggest that AASs increase neuronal vulnerability to an excitotoxic insults and may therefore facilitate neuronal death associated with acute or chronic CNS disorders.