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**Short communication**

# Effects of androsterone on convulsions in various seizure models in mice

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**Abstract:**
It is believed that a deficiency of androgens, including free testosterone, may promote the development of convulsions. The present study revealed differences in the action of androsterone (AND), a major excreted metabolite of testosterone and a neurosteroid, in three commonly used seizure models in mice. AND administered intraperitoneally exhibited dose-dependent protection against tonic-clonic convulsions caused by maximal electroshock (MES) with $ED_{50}$ (effective dose$_{50}$) of 227 mg/kg. The compound also inhibited the convulsive action of pentylenetetrazole (PTZ), increasing its $CD_{50}$ (convulsive dose$_{50}$) for clonic convulsions from 77.2 (PTZ + saline) to 93.9 ($p < 0.05$) for PTZ + AND 40 mg/kg and 113.9 mg/kg ($p < 0.001$) for PTZ + AND 60 mg/kg. In mice pretreated with 60 mg/kg AND, the $CD_{50}$ for PTZ-induced tonic convulsions increased from 102 to 127.6 mg/kg ($p < 0.01$). Surprisingly, doses of 50 and 100 mg/kg AND lowered the $CD_{50}$ for kainate (KA)-induced convulsions from 40.8 to 28.7 ($p < 0.05$) and 25.4 mg/kg ($p < 0.001$), respectively. In summary, for two of the mouse seizure models, our findings confirmed previous studies that demonstrated protective activity of AND. However, the potentiation of KA-induced convulsions by AND was somewhat unexpected and suggested that AND may also possess proconvulsant activity.

**Key words**:
androgens, androsterone, epilepsy, kainate, maximal electroshock, pentylenetetrazole, seizures

## Introduction

Reproductive endocrine disorders that can be attributed to reduced androgen levels (such as subnormal free testosterone), are common in epileptic men [4]. In addition, a deficiency of androgens may promote the development of epileptic discharges and increase seizure susceptibility [3].

Subnormal levels of plasma free testosterone result in reduced production of its major excreted metabolite – androsterone (5α-androstan-3α-ol-17-one, AND). AND has been found in the adult brain, and its action as a neurosteroid with γ-aminobutyric acid $(GABA)_A$-receptor-modulating activity has been proposed [20]. As the role of GABAergic transmission in the pathogenesis of convulsions is crucial [1], alterations in AND levels are relevant to seizure control [18]. Indeed, AND has been found to exert protective activity in both *in vitro* and *in vivo* experiments [5, 7, 10].

In this study, we investigated the effects of AND on convulsions elicited in mice using three commonly accepted seizure models: maximal electroshock (MES), pentylenetetrazole (PTZ) and kainate (KA).

## Materials and Methods

### Animals

The experiments were performed on adult male Swiss mice weighing 20–25 g. The animals were kept under standard laboratory conditions on a natural light-dark cycle, with ambient temperature of 18–22°C, relative humidity of 52–58%, and unlimited access to chow pellets and water. All animals were acclimatized to their home cages for 1 week before testing. The experimental groups, consisting of 8 mice, were chosen by means of a randomized schedule. Each mouse was used only once. The tests were performed between 8:00 and 14:00. The control groups were always tested on the same day as the corresponding experimental groups. The experimental protocol and procedures were followed according to "Principles of Laboratory Animal Care" (NIH publication No. 86–23, revised 1985), approved by the Medical University of Lublin Ethics Committee for the use of experimental animals and confirmed with the European Communities Council Directive (86/609/EEC).

### Drugs

AND was suspended in a 1% aqueous solution of Tween 80, whereas KA and PTZ (all from Sigma, St. Louis, MO, USA) were dissolved in sterile saline for immediate administration, either intraperitoneally (*ip*) at a volume of 10 ml/kg of body weight, or subcutaneously (*sc*) at a volume of 5 ml/kg of body weight. Fresh drug solutions or suspensions were prepared *ex tempore* on each experimental day. Control animals were injected with equivalent amounts of sterile saline or 1% solution of Tween 80 in water using the same route.

### Electroconvulsions

Electroconvulsions were produced with a current delivered *via* ear-clip electrodes by a Rodent Shocker generator (constant-current stimulator Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The criterion for convulsant activity was tonic hindlimb extension (i.e., the hind limbs of animals became extended at 180° to the plane of the body axis). The protective activity of AND against MES-induced convulsions (elicited with 0.2 s stimulus duration and fixed current intensity of 25 mA) was determined.

### Pentylenetetrazole-induced convulsions

Clonic convulsions were induced by *sc* administration of PTZ at doses ranging from 60 to 140 mg/kg. Tonic convulsions were induced by injection of higher doses (90–160 mg/kg) of PTZ. Following injection of the convulsant, mice were placed separately into transparent Plexiglas cages (25 × 15 × 10 cm) and observed for 30 min for the occurrence of seizures. The clonic seizure activity was defined as clonus of all four limbs for at least 5 s, with an accompanying loss of the righting reflex. The tonic seizure was definied as the tonic extension of all four limbs. The number of animals convulsing out of the total number of mice tested was recorded for each treatment condition. An intensity-response curve was constructed from the percentage of convulsing mice. The convulsive action of PTZ was quantified as the $CD_{50}$ (convulsive $dose_{50}$; the dose producing convulsions in 50% of mice). To determine the $CD_{50}$ value, the effects of five different doses of PTZ were tested.

### Kainate-induced convulsions

In the KA model, seizures were induced following administration of the convulsant at doses of 30–60 mg/kg. As described above, mice were placed in cages and observed for 2 h. The limbic convulsions were characterized by repetitive rearing and falling, whole body jerks, occasional opisthotonus, Straub tail, and sometimes explosive running, that progressed into severe clonic movements involving all four limbs, and, in most cases, status epilepticus. The number of convulsing mice out of the total number of mice tested was recorded for each treatment condition. Four groups of mice were injected with various doses of KA and the $CD_{50}$ dose (indicating the convulsive action of KA) was calculated from a dose-response curve with four data points.

### Experimental design

At first, the same dose of AND was injected *ip* into five groups of mice. The duration of AND pretreatment was based on information concerning its biological activity obtained from the literature and confirmed in our pilot experiments. The doses ranged as