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# Deoxycorticosterone's anticonvulsant effects in infant rats are blocked by finasteride, but not by indomethacin

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**Abstract**

Deoxycorticosterone (DOC) is a steroid hormone that suppresses seizures in both humans and animals. At higher doses, DOC's anticonvulsant actions are accompanied by sedation and ataxia. The mechanism of DOC's anticonvulsant actions is not known, although it has been suggested that they may relate to DOC's secondary metabolite 3-alpha-5-alpha-tetrahydrodeoxycorticosterone (THDOC). The present study was designed to study the relation of DOC's anticonvulsant actions to its primary and secondary metabolites in 15-day-old rats. It was found that DOC's anticonvulsant and ataxic effects were suppressed by finasteride, which blocks the formation of DOC's primary metabolite, 3-alpha-5-alpha-dehydrodeoxycorticosterone (DHDOC). They were not suppressed by indomethacin (INDO), which blocks the conversion of DHDOC into THDOC. The direct anticonvulsant effects of DHDOC and THDOC were also tested. DHDOC and THDOC were both potent anticonvulsants in 15-day old rats. Both also caused ataxia at high doses. DHDOC had a therapeutic index (TI) of 3.2, however, which was better than either DOC (TI = 1.2) or THDOC (TI = 1.5). It appears that DOC itself is not anticonvulsant, but that its anticonvulsant effects may relate to both its primary and secondary metabolites. DOC's primary metabolite, DHDOC – with its good TI – deserves a test in the treatment of childhood seizures.


*Keywords:* Deoxycorticosterone; Infant rats; Finasteride; Indomethacin; DHDOC; THDOC; Neurosteroids; Seizures; Anticonvulsant

## Introduction

Deoxycorticosterone (DOC) is a steroid hormone that has been shown to suppress seizures both in humans and in animals (Selye, 1941; McQuarrie et al., 1942; Aird, 1944, 1951; Spiegel and Wycis, 1945; Woodbury, 1952; Craig, 1966; Craig and Deason, 1968; Reddy and Rogawski, 2002; Edwards et al., 2002a,b, 2005).

The mechanism of DOC's anticonvulsant actions is not known, although it has been suggested that they may relate to DOC's secondary metabolite 3-alpha-5-alpha-tetrahydrodeoxycorticosterone (THDOC) (Reddy and Rogawski, 2002). The present study was designed to study the relation of DOC's anticonvulsant actions to its primary and secondary metabolites in 15-day-old rats.

Initial studies involved blockers of DOC's metabolic pathway. DOC's anticonvulsant actions were studied in the absence and presence of finasteride (FIN) or indomethacin (INDO). FIN competitively blocks the 5-alpha reductase enzyme types 1 and 2 (Azzolina et al., 1997), and therefore stops the conversion of DOC into its first metabolite dihydrodeoxycorticosterone (DHDOC) (Poletti et al., 1998) (Materials and methods).

INDO competitively blocks 3-alpha-hydroxysteroid-dehydrogenase (3-alpha-HSD) and therefore stops the conversion of DHDOC into THDOC (Penning et al., 1985; Beyer et al., 1999). It also stops the re-conversion of THDOC back into DHDOC (Materials and methods).

DOC's anticonvulsant actions were therefore studied in the absence or presence of FIN or INDO. In recent experiments, Edwards et al. (2005) have reported that DOC's anticonvulsant effects in infant rats are partially, but not fully, blocked by

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finasteride (FIN). Edwards et al., therefore, concluded that part, but not all, of DOC's anticonvulsant effects were related to its metabolites. Reddy and Rogawski (2002), however, working in adult rats, found that FIN was able to completely block DOC's anticonvulsant actions. They concluded that DOC's anticonvulsant effects relate entirely to its metabolites—at least in adult rats. INDO, which blocks the conversion of DHDOC to THDOC, also partially blocked DOC's anticonvulsant effects in the experiments of Reddy and Rogawski.

The present experiments were designed to continue the study of DOC's anticonvulsant effects in 15-day-old rats. In addition to studying DOC's effects in the absence or presence of FIN or INDO, the direct anticonvulsant effects of DHDOC and THDOC were studied in dose–response experiments. These were conducted in the absence or presence of INDO, which blocks the conversion of DHDOC into THDOC and also the re-conversion of THDOC into DHDOC. Ataxia was scored immediately before each test. Maximal seizures were induced with pentylenetetrazol (PTZ).

## Materials and methods

This research was conducted in accordance with the guidelines of the Canadian Council on Animal Care, and was approved by the Animal Care Committee of the Faculty of Medicine of the University of Toronto.

### *Subjects*

Fifteen-day old male Sprague–Dawley rat pups (Charles River, Canada) served as subjects. Litters of 12 pups were housed with their dams in transparent, plastic cages (24 × 24 × 45 cm) in a vivarium maintained at 21°C on a 12-h light/dark cycle (lights on at 7:00 a.m.). Food (Purina Rat Chow) and water were provided ad libitum. One day before the test day, animals were transported to the test room in their home cages. They were allowed to acclimatize for at least 20 h prior to the experiments. All experiments were performed between 9 and 13 h. After completion of the experiments, subjects were sacrificed by $CO_2$ inhalation followed by cervical dislocation.

### *Drugs*

All steroid hormones, plus INDO, β-cyclodextran, methylcellulose, and PTZ were obtained from Sigma Chemicals (St. Louis, U.S.A). FIN (1,(5alpha)-androstan-4-aza-3-one-17beta-(*N-tert*-butyl-carboxamide)) was obtained from Steraloids Inc. (Newport, U.S.A.).

Hormones were dissolved in a 45% aqueous solution of β-cyclodextran and injected subcutaneously (s.c). FIN was dissolved in 45% aqueous β-cyclodextran, INDO in methylcellulose, and PTZ in physiological saline. FIN, INDO, and PTZ were injected intraperitoneally (i.p.) with an injection volume of 0.1 ml/30 g. Control subjects received volume-matched s.c. or i.p. injections of 45% β-cyclodextran, methylcellulose, or physiological saline.

### *Procedure for the MMT seizure test*

The maximal pentylenetetrazol test (MMT) is a model for tonic–clonic seizures in humans (Fisher, 1989). In adult rats, the presence or absence of tonic hindlimb extension is used as the criterion for scoring the presence or absence of MMT seizures (Fisher, 1989). In infant rats, however, tonic hindlimb extension cannot be reliably elicited in the MMT model (Velisek et al., 1992; Mares and Schickerova, 1980; Mares and Velisek, 1983). Reliable tonic forelimb extension (FLE), however, can be achieved in 2 week old rats (Velisek et al., 1992; Mares and Schickerova, 1980; Mares and Velisek, 1983). In the present studies, therefore, the presence of absence of tonic FLE was also used as the criterion for scoring the presence or absence of MMT seizures.

Subjects were injected i.p. with a dose of 90 mg/kg of PTZ. Following injection, subjects were placed in a test chamber and observed for 30 min. Seizures were scored as 'present' when a FLE of 90° or more was observed.

### *Procedure for DOC dose–response experiments*

Rats received an injection of DOC (10, 20, 30, 40, or 50 mg/kg, s.c.) or β-cyclodextran vehicle (s.c.). Thirty minutes later, PTZ was administered (90 mg/kg, i.p.). Just before the PTZ injection, subjects were scored for ataxia (below). Subjects were then observed for 30 min, and seizures were scored as "present" or "absent".

### *Procedure for DOC dose–response experiments in the absence or presence of finasteride or indomethacin*

It has been shown that FIN is active in both adult (Gao et al., 2002; Phan et al., 1999) and prepubertal rats (Lephart and Husmann, 1993). Previous studies have shown that a FIN dose of 50 mg/kg fully blocks the actions of 5-alpha reductase (Lephart et al., 1996). A dose of 50 mg/kg was therefore used in the present studies. It has also been shown,

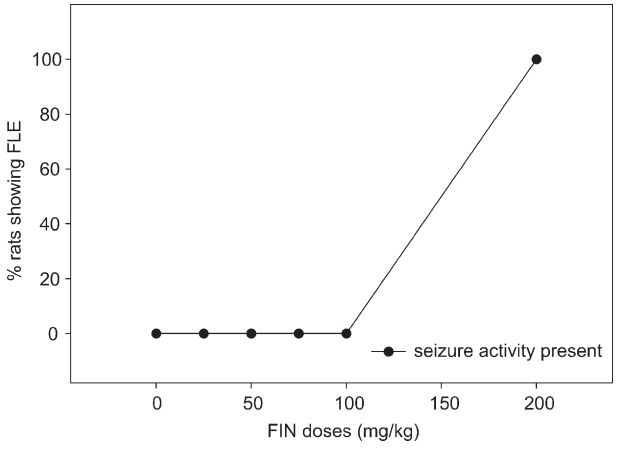


Fig. 1. Finasteride's proconvulsant effects of FIN in 15-day-old rats. Rats were injected with FIN (0, 25, 50, 75, 100, or 200 mg/kg, i.p.) and observed for 90 min. The occurrence of seizure activity (FLE) was scored ($N = 6$ rats per point).