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Research Report

Anticonvulsant actions of deoxycorticosterone

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ABSTRACT

Purpose: Adrenocorticotrophic hormone (ACTH) suppresses several types of childhood seizures, but it has many side effects. The mechanism of ACTH's anticonvulsant actions is not known. ACTH, however, releases deoxycorticosterone (DOC) – as well as cortisol – from the adrenal cortex and it has been suggested that DOC may mediate, at least in part, ACTH's anticonvulsant actions. The present study assessed DOC's anticonvulsant actions in infant rats. Age-related changes in DOC's anticonvulsant actions were also studied. Methods: DOC's anticonvulsant actions were assessed against hippocampal-kindled, maximal pentylenetetrazol test (MMT) and maximal electroshock (MES) seizures in 15-day-old rats. Age-related changes in responsiveness to DOC were also assessed using the MMT model. Results: DOC suppressed generalized convulsions in all three of the seizure models. Focal spiking in the hippocampal-kindling model, however, was not fully suppressed, even at high doses. Ataxia increased proportionally with the dose, with the time of peak seizure suppression roughly correlating with the time of peak ataxia in all models. DOC was anticonvulsant in both infant and adult rats. ED50s, however, were much higher in adults. Young rats showed ataxia at the time of testing (15 min), whereas adult rats did not, although ataxia was seen at later times. Conclusions: DOC is a potent anticonvulsant against generalized seizures, particularly in infants. It deserves a clinical test against generalized seizures in infants.

1. Introduction

ACTH suppresses several types of childhood seizures (Baram et al., 1996; Hrachovy, 2002; Hurts, 1994; Schlumberger and Dulac, 1994; Snead et al., 1989). ACTH therapy, however, results in numerous and serious side effects. These include hypertension, immunosuppression, intracranial hemorrhage, and cataracts (Dehkharghani, 1993).

To date, the mechanism of action of ACTH's anticonvulsant effects is unknown (Holmes and Vigevano, 1997; Rogawski and Reddy, 2002). ACTH, however, acts on the adrenal cortex to induce the release of several steroid hormones *in addition* to corticosterone (CORT). These include progesterone and deoxycorticosterone (DOC) (Schimmer and Parker, 2001). In animal models, neither ACTH nor CORT has significant anticonvulsant actions (Edwards et al.,

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Abbreviations: ACTH, adrenocorticotriphic hormone; AD, afterdischarge; ADT, afterdischarge threshold; CO_2, carbon dioxide; CORT, corticosterone; DHDOC, dihydrodeoxycorticosterone; DOC, deoxycorticosterone; EEG, electroencephalograph; ED50, a dose that produced 50% of the maximal effect; FLC, forelimb clonus; FLE, forelimb extension; FLF, forelimb flexion; MES, maximal electroshock seizure; min, minutes; MMT, maximal pentylenetetrazol seizure; PTZ, pentylenetetrazol; THDOC, tetrahydrodeoxycorticosterone

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2002a; Thompson and Holmes, 1987). Two of the steroids released with CORT, however, do have anticonvulsant effects: (1) progesterone (Edwards et al., 2002a; Frye et al., 2002; Frye and Scalise, 2000; Kokate et al., 1999); and (2) DOC (Aird, 1944, 1951; Craig, 1966; Craig and Deason, 1968; Edwards et al., 2002a,b; McQuarrie et al., 1942; Reddy and Rogawski, 2002; Selye, 1941; Spiegel and Wycis, 1945; Woodbury, 1952).

These data have led to the DOC hypothesis of ACTH's mechanism of action (Edwards et al., 2002a; Rogawski and Reddy, 2002). According to this hypothesis of DOC's action, stimulation of the adrenal zona fasciculata by ACTH results in DOC synthesis and release (Kater et al., 1989; Tan and Mulrow, 1975). DOC then breaks down into two neurosteroid metabolites, dihydrodeoxycorticosterone (DHDOC), and tetrahydrodeoxycorticosterone (THDOC) (Reddy and Rogawski, 2002; Schambelan and Biglieri, 1972). These neurosteroids are strong modulators of the $GABA_A$ receptor (Lambert et al., 1995; Majewska et al., 1986; Reddy and Rogawski, 2002). They increase GABA-ergic inhibition, which is thought to cause sedation and stop seizures (Belelli et al., 1990; Czlonkowska et al., 2000; Devaud et al., 1995; Edwards et al., 2002b; Kokate et al., 1994, 1996; Reddy and Rogawski, 2002).

In previous dose–response studies from our laboratory – involving infant rats – Edwards et al. (2002b) showed that DOC was able to suppress forelimb flexion (FLF) and forelimb clonus (FLC) in the maximal pentylenetetrazol test (MMT) seizure model at relatively low doses (ED50 for FLF was 5 mg/kg, s.c.). Higher doses of DOC were also able to suppress forelimb extension (FLE) in the maximal electroconvulsive shock (MES) model (ED50 was 20 mg/kg, s.c.). A single-dose-study suggested that DOC might also be effective in the kindling model, but dose–response testing was not done (Edwards et al., 2002a). Time–response testing was also not done, and toxicity was not assessed in any of these experiments, which made it impossible to calculate therapeutic indices (TIs).

Edwards et al. (2002b) also reported that DOC's anticonvulsant actions in rats were lost after puberty. These data, however, conflict with the report of Reddy and Rogawski (2002), who found that DOC suppresses seizures in adult rats.

The present study continued the investigation of DOC's anticonvulsant actions in infant rats. DOC's anticonvulsant actions were tested in 15-day-old rats in three different seizure models: the hippocampal-kindling model, the MES model, and the MMT model (Experiment 1). Neural development in 15-day-old rats resembles that of human infants within the first year of life (Edwards et al., 2002b; Hobbing and Sands, 1995). As in Edwards et al., a 15-min injection-test interval was used. Ataxia was rated just before the seizure tests using Loscher's ataxia scale, and TIs were calculated. Time–response testing was also done in each model to assess the duration of DOC's anticonvulsant effects. Dose–response studies with rats of different ages were done in the MMT model to address the question of whether DOC has anticonvulsant effects in adult rats (Experiment 2). The MMT model was chosen because it had previously been used by Edwards et al. (2002b).

2. Results

2.1. *Experiment 1: dose– and time–response studies*

2.1.1. *Kindled seizures*

The data related to DOC's anticonvulsant effects against hippocampal-kindled seizures are presented in Fig. 1. DOC suppressed *generalized* convulsions in hippocampal-kindled subjects with an ED_{50} of 10 mg/kg. Complete suppression of these seizures was seen at higher doses. Ataxia data for these subjects are presented in Fig. 2. Ataxia increased proportionally with dose. The TD_{50} was 20 mg/kg and the TI for generalized convulsions was 2.0.

DOC was unable to *fully* suppress hippocampal ADs, however, even at the highest dose tested (DOC=80 mg/kg) (Fig. 1). The ED_{50} was 80 mg/kg. As noted above, the TD_{50} for these subjects was 20 mg/kg. The TI, therefore, was 0.25.

The time–response data related to DOC's suppression of generalized kindled convulsions are presented in Fig. 3. DOC suppressed over 60% of generalized convulsions 10 min post-injection, and 100% at 30 min. DOC's effects started to decrease by 1 h and had disappeared by 2 h. The related ataxia data are presented in Fig. 4. Ataxia paralleled seizure protection, with a slight time lag.

2.1.2. *MES seizures*

The dose–response data related to DOC's anticonvulsant effects on MES seizures are presented in Fig. 1. DOC suppressed MES seizures with an ED_{50} of 30 mg/kg. At higher doses, there was a complete suppression of FLE. Data related to ataxia are presented in Fig. 2. The TD_{50} was 25 mg/kg and the TI was 0.83.

The time–response data related to DOC's anticonvulsant effects appear in Fig. 3. In these tests, 50% protection was observed at 10 min post-injection, and 100% protection was seen at 20 min. DOC's effects started to decrease by 30 min, but there was still some protection even 3 h post-injection. The

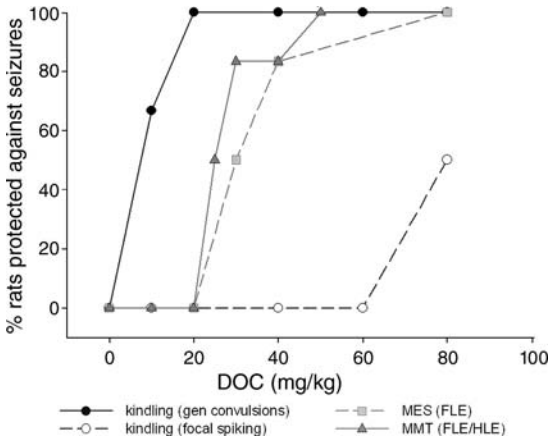

Fig. 1 – Dose–response data for DOC's anticonvulsant effects in 15 day-old rats against hippocampal kindled, MES and MMT seizures. Different doses of DOC were administered to pups, followed 15 min later by the seizure provoking stimulus. (Ataxia scores for these subjects appear in Fig. 2.)