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Effects of 18-hydroxydeoxycorticosterone on central nervous system excitability

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Summary. The effects of 18-hydroxydeoxycorticosterone (18-OH-DOC) on central nervous system excitability were studied in adrenalectomized rats. Sixty-four evoked potentials (EP) recorded from the pontine reticular formation were averaged before and after the injection of vehicle and hormone. 750 µg of 18-OH-DOC dissolved in 0.5 ml of a 4:1 saline Cremophor-EL solution were injected i.v. A decrease of 55.7 ± 6.1% in the amplitude of the EPs was observed with the hormone 16.3 min ± 2.7 (SE) after injection. Amplitude values returned to baseline levels 38 min ± 6.8 (SE) after injection. The secretion of 18-OH-DOC is greatly increased by ACTH and might modulate central nervous system function.
Key words. 18-OH-DOC; brain; excitability.

Nervous systems are sensitive targets for steroid hormones[1,2]. Thus, besides their well-known effects on neuroendocrine feedback mechanisms, steroid hormones modulate CNS excitability and affect mood and sexual behavior[3]. Within the adrenal steroids the glucocorticoid type hormones have received the most attention in the past.

Recent work has demonstrated that mineralocorticoid type hormones are taken up and metabolized by nervous tissue[4]. Moreover, DOC, as well as its ring-A reduced metabolites DHDOC and THDOC significantly reduced brain excitability in the rat[5]. DOC, however, is not the only mineralocorticoid hormone in the rat. The rat adrenal is unique in secreting, as its second most prominent steroid after corticosterone, 18-OH-DOC[6]. 18-OH-DOC is a sodium-retaining compound produced mainly in the zona fasciculata of the adrenal cortex under the control of ACTH[6]. Radiochemical studies, as well as measurement of the endogenous hormone, revealed that 18-OH-DOC is widely distributed in the brain[7,8].

We therefore thought it appropriate to examine the possible effects of 18-OH-DOC on brain excitability.

Methods. Adrenalectomized male Sprague-Dawley rats weighing 250–300 g were used. Adrenalectomies were carried out in our laboratory 2 days prior to the experiments and the rats were maintained on Purina Chow and physiologic saline ad libitum. The rats were anesthetized with urethane (1.1 g/kg) given i.v. under ether anesthesia. A trachea cannula was positioned and the femoral artery and vein were cannulated. The femoral arterial pressure and the percentage CO_2 in the expired air were monitored throughout the experiments with a Statham P23 pressure transducer and a Godart Statham capnograph, connected to the bridge mode of a Grass DC pre-amplifier respectively. Expired CO_2 was maintained at 3.8–4%. The animals were paralyzed with gallamine triethiodide (Flaxedil, Poulenc) 10 mg/kg and maintained under artificial respiration. The rectal temperature was servoregulated to 37 °C with a Yellow Springs Instrument feedback system attached to a DC heating source. In preparation for recording, a bone flap was removed between the lamboidal suture and bregma and the dura was retracted. The sciatic nerve contralateral to the recording site was prepared for stimulation and maintained in a pool of warm paraffin oil.

Field activity was recorded from bipolar electrodes stereotaxically positioned in regions corresponding to the pontine reticular formation. Evoked potentials were amplified through a Tektronix 122 preamplifier (band width of 0.2 Hz to 10 kHz). After further amplification, the potentials were displayed on a Tektronix 565 oscilloscope. They were averaged with an Enhancetron digital memory oscilloscope (Nuclear Data Inc.) and displayed on a Tektronix 5000 series oscilloscope and photographed from it. Anodal electrolytic DC lesions, 20 µa for 15 s, were made for histological verification of recording sites. 18-OH-DOC was dissolved in a 4:1 saline:cremophor-EL solution.

(Cremophor EL, a polyoxyethylated castor oil, was purchased from Sigma). Only one injection (750 µg/0.5 ml) was given in each experiment to avoid dose summation. The injection was

Evoked potential changes following 18-OH-DOC administration

Mean baseline amplitude N = 7	Mean amplitude after injection N = 7	Time of peak decrease N = 7*	Recovery time N = 6*	Amplitude recovery N = 6*
79.9 μv ± 21.0 (S.D.)	35.0 μv ± 14.8 (S.D.)	16.3 min ± 2.7 (S.D.)	38.0 min ± 16.7 (S.D.)	67.0 μv ± 24.2 (S.D.)

Before – After μV: 44.9 ± 7.2 (SE) paired, $p < 0.001$ ($t = 6.24$, $df = 6$); Before – After %: 55.7 ± 6.1 (SE) paired, $p < 0.001$ ($t = 9.13$, $df = 6$).
*One animal died before recovery.

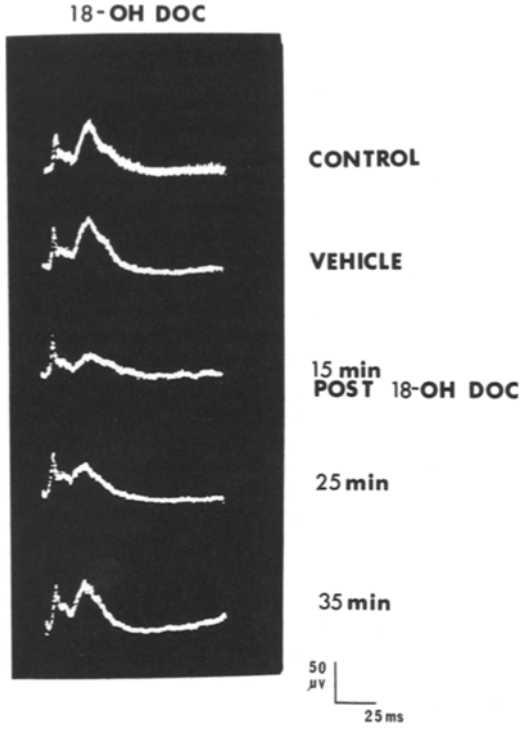
given over a period of 1.5 min. No change in blood pressure and PCO_2 was produced by this slow administration of hormone or vehicle.

Results. Electric stimulation of the sciatic nerve elicited a response in pontine brain stem regions. Characteristically, one fast and one slow component were observed. The fast component peaked at 8–10 ms and the slow from 20 to 25 ms.

Five control sets of 64 averaged potentials were observed which served as the baseline reference. These were compared with five sets of averaged potentials recorded after a control injection of the steroid vehicle alone. In 7 out of 8 animals 18-OH-DOC produced a decrease in the amplitude of the evoked responses. In the remaining animal, no change in the evoked potential was noted after the hormone injection. The decrease was consistently observed in the slow, late component of the response (fig.). The table shows the effects of 18-OH-DOC in terms of latency, effect on amplitude and recovery time of the late evoked potentials. Peak decrease occurred at 16.3 min ± 2.7 (SE) with average return to within baseline level amplitudes at 38.0 ± 6.8 min.

Histological examination of recording sites showed all of them to be within the confines of pontine reticular formation regions.

Discussion. It is known that 18-hydroxylation modifies certain biological properties of some pregnane derivatives[9]. The results presented, however, show that 18-hydroxylation does not affect the well-established effect of DOC in decreasing brain excitability[5]. Thus 18-OH-DOC significantly reduced brain stem responses to sciatic stimulation in 7 out of 8 animals studied.



A representative sample of the effect of 18-OH-DOC on sciatic evoked potentials recorded from pontine reticular formation sites. Each trace is an average of 64 sweeps.

Secretion rates of 18-OH-DOC, obtained by adrenal vein cannulation, were found in earlier studies to range from 46 to 126 μg per 100 g rat per hour[10]. The chosen dose could therefore be secreted under maximal stress in 2–6 h and thus be within physiological range even if the likelihood of metabolic degradation before access to the CNW were discounted. The effects of different doses and, equally important, of different combinations of steroids on CNS excitability remain to be assessed.

In the human 18-OH-DOC is relatively more responsive to ACTH than is cortisol and in cases of hypersecretion of ACTH, e.g. Cushing's Syndrome, it may be secreted in milligram amounts[11]. The time course of the response to 18-OH-DOC noted in this study and by others on steroid effects upon the CNS[5,12,13], suggest a steroid-cell interaction at non-nuclear sites and, thus, distinct from the genomic effects attributed to classical mineralocorticoid and glucocorticoid action. Such non-genomic steroid effects deserve consideration in the context of normal and abnormal adrenocortical function. The hypersecretion of 18-OH-DOC noted in Cushing's Syndrome might in this manner feature in the mental disturbances so frequently associated with the disease. That 18-OH-DOC might be of physiological or pathophysiological significance in the interaction with the CNS is rendered attractive by the low affinity for corticosteroid-binding globulin of this hormone, in contrast to that of cortisol, and by the relatively greater lipid solubility, factors which might be expected to favor more ready access to the CNS.

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