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19-NOR-DOC BIOSYNTHESIS IN THE ISOLATED PERFUSED RAT KIDNEY*

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Summary—19-nor-deoxycorticosterone (19-nor-DOC) is a potent salt retaining and hypertensinogenic mineralocorticoid that is excreted in the urine. While the precursor of 19-nor-DOC, 19-oxo-DOC, is produced by the adrenal cortex, conversion to 19-nor-DOC does not occur in the adrenal gland. We have examined the hypothesis that 19-nor-DOC is synthesized from precursors in the kidney. 19-oxo-DOC was added to the perfusate of isolated rat kidney preparations ($n = 5$) at a concentration of 10 μM. During 1 h of perfusion following addition of 19-oxo-DOC, 71 $\pm$ 6% of the precursor was converted to 19-oic-DOC, an immediate precursor of 19-nor-DOC, and 8.3 $\pm$ 1.8% was converted to 19-nor-DOC. This represents the first definitive evidence that 19-nor-DOC is produced in the kidney from adrenal precursors.

19-nor-deoxycorticosterone (19-nor-DOC) was first isolated from the urine of rats with adrenal regeneration hypertension [1]. Since its isolation in human urine [2], interest has markedly increased in this compound and in its biosynthesis and biologic activities. 19-nor-DOC is a potent mineralocorticoid with a high affinity for mineralocorticoid receptors [3, 4]. It is hypertensinogenic in rats, has a sodium-retaining activity similar to that of aldosterone and is independent of the renin–angiotensin system [5]. 19-nor-DOC has not been isolated from adrenal incubation media [6]. It is believed that it is produced in the periphery from adrenal precursors. 19-hydroxy-DOC, 19-oxo-DOC, and 19-oic-DOC serve as precursors of 19-nor-DOC [6]. In normal humans 19-nor-DOC is present in the urine but it is not detectable in plasma. The absence of tetra-hydro-19-nor-DOC, a hepatic metabolite in the urine, also argues against the circulation of 19-nor-DOC, since this metabolite is present when 19-nor-DOC is infused intravenously. Thus available evidence suggests that 19-nor-DOC is synthesized in the kidney from circulating precursors. The purpose of this study is to test this hypothesis. The isolated kidney perfused *ex vivo* provides a useful model for studying this issue.

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MATERIALS AND METHODS

Kidney perfusion

Male Sprague–Dawley rats, weighing between 250 and 350 g, fed on regular Purina rat chow (Ralston Purina, Chicago, Ill.) and allowed free access to water were used for all experiments. Rats were anesthetized with pentobarbital sodium (Nembutal, 5 mg/100 g body wt i.p.). Mannitol (200 mg i.v.) was given, before the right ureter was cannulated with PE-50 tubing. Heparin (100 U i.v.) was given before cannulation of the renal artery. Perfusion of the right kidney was performed using a red cell free perfusate as previously described [7]. The kidneys were perfused at a constant renal arterial pressure of 95–105 mmHg (arterial line pressure corrected for cannula resistance). The perfusion medium consisted of a Krebs–Henseleit buffer containing 4.5 g/100 ml of dialyzed bovine serum albumin (Sigma, St Louis, Mo.), 5 mM glucose, 2.5 mM calcium, and amino acids, as previously described [7]. The system consists of reperfusion of the urinary and venous effluents into the perfusion medium which is used to measure the steroid. After an equilibration period of 30 min, the 19-oxo-DOC (courtesy Gomez-Sanchez CE, Tampa, Fla) was added to the perfusate at a concentration of 10 μM. Aliquots of the perfusate were obtained at three 20 min intervals. The glomerular filtration rate

(GFR) was determined by inulin clearance using (methoxy-^{3}H) inulin as previously described [7]. The GFR ranged between 0.5 and 0.6 ml/min and the renal perfusate flow rate between 22–30 ml/min. In control experiments ($n = 3$), 19-oxo-DOC was added to perfusate at 37°C, and at a comparable concentration (10 μM), but without kidney perfusion. No 19-oic-DOC or 19-nor-DOC were detected at 0, 20, 40, or 60 min of incubation.

Steroid assay

The perfusate samples were extracted with methylene chloride and were reduced to dryness *in vacuo*. The material was chromatographed on a celite plate and subsequently dried and developed. 19-nor-DOC standard and antibodies were provided by Gomez-Sanchez CE (Tampa, Fla). 19-nor-DOC was measured by radioimmunoassay method as previously described [8]. The interassay variability was 9.8%, the cross-reactivity of 19-nor-DOC antibodies with cortisol, 19-oxo-DOC, androstenedione, DHEA and testosterone was less than 0.1%, whereas the cross-reactivities with 19-oic-DOC, corticosterone, 11-deoxycortisol, progesterone and DOC were 0.75, 0.2, 0.2, 3.9 and 13.5%, respectively. Since 19-nor-DOC and DOC completely separated on the celite chromatography plate there was no interference by DOC with RIA despite the considerable cross-reactivity of the two compounds with 19-nor-DOC antibody.

After extraction, as previously described [17], the perfusate was reduced to dryness and acetylated overnight in acetic anhydride–pyridine (2:1, vol/vol). After the addition of 2 ml of 95% ethanol, the acetate was dried under nitrogen and the residue subjected to HPLC (DuPont, Delaware), with a C18 reverse-phase column. Acetonitrile–water (34:66, vol/vol) was used for elution. The 19-oic-DOC–acetate was further purified by an additional HPLC step using acetonitrile–water (30:70, vol/vol) for elution. 19-oic-DOC–Ac exhibited similar running rates as a standard provided by MLD Pharmaceutical Institute, Cincinnati, Ohio. 19-oic-DOC was estimated according to the area under the curve.

Statistics

All data are expressed as the mean ± SE. Data were analyzed by two-way analysis of variance (ANOVA) followed by a Scheffé's test. For multiple comparison, a $P < 0.05$ was considered significant.

RESULTS

19-nor-DOC concentration increased progressively in the perfusate to reach concentrations of 38.6 ± 5.4 ng/ml, 216.2 ± 38.7 ng/ml and 262.8 ± 56.3 ng/ml at 20, 40 and 60 min, respectively. Maximum concentration of 19-nor-DOC was reached by 40 min (Table 1).

At the end of the perfusion, the concentration of 19-oic-DOC was 7.14 ± 0.63 μM (2.5 ± 0.2 μg/ml) and the concentration of 19-nor-DOC was 0.83 ± 0.18 μM (263 ± 56 ng/ml).

The proportion of 19-oxo-DOC converted to 19-oic-DOC (71.4 ± 6.0%) and to 19-nor-DOC (8.3 ± 1.8%) by the end of the hour of perfusion is shown in Table 1.

DISCUSSION

19-nor-DOC has been shown to be markedly hypertensinogenic in the rat by Hall *et al.* [9]. Griffing *et al.* [10] also demonstrated an increase in 19-nor-DOC in some patients with hypertension due to primary aldosteronism. Kagawa and Van Arman [11] found the sodium-retaining activity of 19-nor-DOC to be between 2 and 5 times that of deoxycorticosterone itself. Gomez-Sanchez has shown that 19-OH-DOC, 19-oxo-DOC, and 19-oic-DOC serve as precursors of 19-nor-DOC. Dale *et al.* [2] identified 19-hydroxy-DOC (19-OH-DOC) in the incubation medium of nucleated rat adrenal glands in the early sodium-retaining phase at a time when 19-nor-DOC has been isolated from urine. However, 19-nor-DOC could not be isolated from adrenal rat incubation media. Thus, while the adrenal gland initiates the first steps in 19-nor-DOC biosynthesis, the final step, the 19-demethylation of DOC has been hypothesized to occur in the kidney. This supposition was always based on several observations. First plasma 19-nor-DOC levels are nearly undetectable; second, when 19-nor-DOC is infused

Table 1. Time-dependent appearance of 19-nor-DOC (±SE) in the perfusate of the isolated kidney ($n = 5$) perfused with 19-oxo-DOC at 10 μM (3.5 μg/ml)

Isolated rat kidney perfused with 19-oxo-DOC			
19-oxo-DOC production		19-oxo-DOC conversion at 1 h	
Time (min)	19-nor-DOC in perfusate (ng/ml)	Steroid produced from kidney perfusion with 19-oxo-DOC	% conversion
20	37 ± 5		
40	216 ± 39*	19-oic-DOC	71.4 ± 6.3
60	262 ± 56*	19-nor-DOC	8.3 ± 1.8

* = $P < 0.05$ compared to 20 min time interval. Percentage of precursor (19-oxo-DOC) converted to 19-oic-DOC and to 19-nor-DOC at 1 h of perfusion ($n = 5$).

intravenously, hepatic metabolites, not normally present, appear in the urine; and finally, adrenal glands incubated *in vitro* do not produce 19-nor-DOC [6, 12–14].

In this study, we have shown that 19-nor-DOC and 19-oic-DOC are synthesized in the kidney from 19-oxo-DOC. 19-oxo-DOC is converted to 19-oic-DOC at a rate of 71% over 1-h period of perfusion. 19-oxo-DOC conversion to 19-nor-DOC is around 8% over the same period of time. While we have demonstrated that the perfusion of an isolated rat kidney with 19-oxo-DOC results in c-19 demethylation to yield to 19-oic-DOC and 19-nor-DOC, we are still uncertain whether this reaction is catalyzed by a specific c-19 desmolase enzyme.

19-nor-DOC and 19-oic-DOC appear to be other examples of extra glandular hormonogenesis comparable to other hormone systems which are synthesized peripherally such as estrogen, Vitamin D, triiodothyronine, dihydrotesterone and progesterone. 19-nor-DOC and 19-oic-DOC are the only known mineralocorticoids that are produced in the target organ (the kidney). 19-nor-DOC is also unique in that it is largely influenced by coticotropin secretion [15–18]. Griffing *et al.* [10] have demonstrated that corticotropin enhances the production of 19-nor-DOC while dexamethasone suppression of corticotropin reduces it. Alterations in sodium balance have a much lesser effect [19]. Thus 19-nor-DOC may be a "stress" mineralocorticoid that acts as a sodium-retaining hormone at its site of production within the kidney and it may play an important role in the pathogenesis of hypertension. 19-oic-DOC was found to be elevated in plasma of a patient with 17-α-hydroxylase deficiency and hypertension [17], but its mineralocorticoid activity is still unknown.

In summary, we have provided the first direct evidence for the synthesis of 19-nor-DOC from its precursors in the kidney.

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