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# Effects of Angiotensin II on Steroid Metabolism and Hepatic Blood Flow in Man

FRANZ H. MESSERLI, WOJCIECH NOWACZYNSKI, MASANOBU HONDA, JACQUES GENEST,

ROGER BOUCHER, OTTO KUCHEL, AND JOSÉ MANUEL ROJO-ORTEGA

**SUMMARY** Metabolic clearance rates (MCR) of aldosterone, cortisol, 11-deoxycorticosterone (DOC), corticosterone, and progesterone were simultaneously measured by constant infusion in eight control subjects before and during angiotensin II infusion in subpressor (3 ng/min per kg) and pressor (22 ng/min per kg) doses. Plasma levels of aldosterone and cortisol, the heat-labile protein-bound fraction of aldosterone, and hepatic blood flow (HBF) (as estimated by the fractional clearance of indocyanine green) were determined concomitantly. Angiotensin II in a subpressor dose produced a significant decrease of the MCR of aldosterone (by 23%), cortisol (by 16%), DOC (by 26%), corticosterone (by 14%) and progesterone (by 33%). The pressor dose

further decreased the respective MCR by 37%, 21%, 40%, 28%, and 42% of the baseline value. Plasma aldosterone levels rose by 317% with subpressor and by 434% with pressor doses. HBF decreased by 18% with subpressor and by 33% with pressor doses of angiotensin II. Furthermore, there were significant negative correlations between the MCR of each steroid and the respective values of the fractional clearance of indocyanine green. We conclude that angiotensin II, by its vasoconstrictive action on the splanchnic vascular bed, decreases the MCR of aldosterone, cortisol, DOC, corticosterone, and progesterone. This decrease has to be taken into account when considering the stimulatory effect of angiotensin II on various plasma steroid concentrations.

ANGIOTENSIN II is the predominant stimulator of aldosterone production by the adrenal cortex.<sup>1,3</sup> Slight and variable stimulatory effects on other steroids have been reported.<sup>4,7</sup> Furthermore, angiotensin II can stimulate ACTH release by direct action on the anterior pituitary.<sup>8</sup> Angiotensin II, however, also produces vasoconstriction of the splanchnic vascular bed and thereby diminishes hepatic blood flow (HBF).<sup>9,10</sup> This pathophysiological mechanism should result in a decrease in the hepatic clearance of those steroids which are extracted to a high degree by the liver,<sup>11</sup> and consequently might contribute to an increase in their plasma levels.

The present study was designed to investigate the effect of angiotensin II in subpressor and pressor doses on the metabolic clearance rates (MCR) of aldosterone, cortisol, 11-deoxycorticosterone (DOC), corticosterone, and progesterone. Plasma levels of aldosterone and cortisol, the heat-labile protein-bound fraction of aldosterone, and HBF as estimated by the clearance of indocyanine green, were determined concomitantly.

## Methods

## EXPERIMENTAL DESIGN

Eight healthy subjects (six male and two female), 19-36 years in age, volunteered for the study. Informed consent had been obtained after the subjects were thoroughly

acquainted with the experimental procedure. Complete physical examination and routine laboratory tests (plasma sodium, potassium, calcium, BUN, uric acid, cholesterol, total protein, albumin, bilirubin, creatinine, alkaline phosphate, LDH, SGOT, hemoglobin, hematocrit, WBC, and urine analysis) were within normal limits. The subjects were admitted the evening prior to the experimental day and went to bed before 10 p.m. They were not on a specific diet, but food with high sodium content, tea, coffee, alcoholic drinks, and smoking were prohibited. Urinary sodium excretion during the 24 hours before the investigation was  $147 \pm 13$  (mean  $\pm$  SEM) mEq, with a range of 108-192. A recumbent position was maintained for 15 hours from the preceding evening until the end of the experiment. The subjects did not receive any food throughout the period of recumbency and the ECG and arterial pressure were monitored continuously (Pressurometer, type 1900, Avionics Research Products).

A priming dose of about 5  $\mu$ Ci of 1,2-<sup>3</sup>H-labeled aldosterone, DOC, corticosterone, cortisol, and progesterone in 10 ml of heterogenous human plasma (supplied by Red Cross and tested for hepatitis-associated antigen) was injected intravenously at about 8 a.m. One hour later a continuous 4-hour infusion at a constant rate was begun with an infusion pump (Sage Instruments) fitted with a 50-ml disposable syringe. The syringe contained a solution of all five labeled steroids in the same plasma. Infusion rate was approximately 2.5  $\mu$ Ci/hour for each steroid. Syringe and infusion pump were calibrated after each procedure. Blood (20 ml) was drawn from an indwelling needle in a cubital vein of the opposite arm into heparinized Vacutainers (Becton, Dickinson) 90 and/or 100 minutes and/or 110 minutes after the beginning of the infusion. At least two samples were drawn from every subject. For four subjects three or four determinations were made. The mean of these values is referred to in the following sections as the baseline (=0) sample. A constant infusion of angio-

From the Steroid Research Department, Clinical Research Institute of Montreal, Quebec, Canada.

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Dr. Messerli's present address is: Medical Policlinic, University Medical School, Berne, Switzerland.

Address for reprints: W. Nowaczynski, D.Sc., Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7.

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otensin II (Hypertensin, Ciba) at a subpressor dose of  $3.0 \pm 1.3$  (mean  $\pm$  SEM) ng/kg per min was started immediately thereafter for 1 hour. The dose was then increased to  $22 \pm 4.8$  ng/kg per min in order to increase diastolic pressure by 20–25 mm Hg. This level was maintained for 70 minutes. Blood was drawn 60, 120, and 130 minutes after the start of the angiotensin II infusion. In one subject a control experiment was performed: the subpressor and pressor doses were infused for 2 hours, respectively, and blood was drawn 20, 10, and 0 minutes before and 60, 80, 100, 120 (subpressor dose), 180, 200, 220, and 240 minutes after the start of the angiotensin II infusion. The samples were immediately centrifuged at 4°C and the plasma was frozen on solid carbon dioxide and stored at  $-20^{\circ}\text{C}$  until the time of the assay.

#### METABOLIC CLEARANCE RATES

MCR was measured as previously reported for aldosterone:<sup>12</sup> 50  $\mu\text{g}$  of carrier and 300 counts/min of  $^{14}\text{C}$ -labeled indicator of all five steroids and 0.5 ml of 1 N NaOH were added to 10 ml of the patient's plasma and to the injected solution. The mixture was extracted with dichloromethane and washed with NaOH, acetic acid, and water.<sup>13</sup> After evaporation the dried extract was chromatographed in an isooctane-benzene-methanol-water (1:2:4:1) system for 4 hours. The respective retardation factor ( $R_f$ ) values for aldosterone, cortisol, corticosterone, DOC, and progesterone were 0.07, 0.15, 0.35, 0.79, and 0.92. Aldosterone and cortisol were further separated by a cyclohexane-benzene-methanol-water (1:9:6:4) chromatography for 8 hours (ratio of cortisol to aldosterone = 1.6).

DOC, corticosterone, and progesterone were rechromatographed in the Bush B<sub>3</sub>, Bush B<sub>5</sub>, and Bush A systems, respectively, for 4 hours. The final dried extract was transferred to counting vials. Calculation of MCR and correction for losses were performed as previously reported.<sup>12</sup>

#### HEPATIC BLOOD FLOW

HBF was estimated three times by a single injection of indocyanine green before, and 40 minutes and 100 minutes after, the start of the angiotensin infusion. Indocyanine green (50 mg) was rapidly injected into an antecubital vein and a 2-ml sample of blood was drawn at 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 minutes after the injection. Indocyanine green concentrations in the serum were estimated with a Beckman DU-2 spectrophotometer at a wavelength of 805 nm. Concentrations were read off a standard calibration curve prepared with solutions of known concentrations of the dye made up in pooled human serum. Fractional clearance was calculated using the method of the least squares for the "ln" (natural logarithms) values of the serum concentrations.

#### STERIODS

Plasma levels of aldosterone were measured by radioimmunoassay<sup>13</sup> and of cortisol by competitive protein binding<sup>14</sup> in the same plasma samples. For calculation of the recoveries of all steroids, the counts of the infused  $^3\text{H}$ -labeled steroids were taken into account. Secretion rates

of aldosterone and cortisol were calculated from the plasma level and the MCR according to the formula:  $\text{SR} = \text{MCR} \times \text{P}/1,000$ , where SR = secretion rate ( $\mu\text{g}/24$  hour) and P = plasma concentration ( $\mu\text{g}/\text{ml}$ ).

Heat-labile protein-bound fractions of aldosterone were determined as previously reported.<sup>15</sup>

#### STATISTICAL ANALYSIS

Statistical comparisons between the baseline and the values during angiotensin II infusion were calculated by an analysis of variance for single factor experiments having repeated measures on the same elements. Comparisons a posteriori between samples from different times were performed by Dunnett's *t*-test. A linear regression was calculated between the MCR of all five steroids and the fractional clearance of indocyanine green.

#### Results

The highest mean coefficient of variation for the MCR baseline values of each steroid was  $5.5 \pm 1.4$  (SD) in four of the eight subjects in whom more than two determinations were made, indicating that a steady state had been reached before the angiotensin infusion. Intraindividual variation of the baseline values of plasma aldosterone and cortisol was smaller than 18% and 12%, respectively, indicating that the concentration of these hormones was quite stable at zero time.

Angiotensin II in subpressor dose decreased the MCR of aldosterone by 23%, of cortisol by 16%, of DOC by 26%, of corticosterone by 14%, and of progesterone by 33% (Table 1). The pressor dose decreased the MCR by 37% (range, 31–54%), 21% (0–48%), 40% (23–56%), 28% (14–37%), and 42% (27–54%), respectively, from the baseline value. Intraindividual variation of the MCR between 120 and 130 minutes were small (less than 7.2%). In the control experiment, the respective MCR of aldosterone (in liters/24 hours per  $\text{m}^2$ ) was 847 (–20 minutes), 843 (–10 minutes), 856 (0 minutes) before the angiotensin II infusion, 683 (60 minutes), 695 (80 minutes), 668 (100 minutes), 676 (120 minutes) with the subpressor dose and 525 (180 minutes), 511 (200 minutes), 543 (220 minutes), 532 (300 minutes) with the pressor dose. This indicates that a new equilibrium has been reached after 60 minutes of angiotensin II infusion which is not systematically affected by prolongation of both infusions for an additional 60 minutes.

Plasma aldosterone levels rose by 317% with subpressor and 434% with pressor doses of angiotensin II. Concomitantly, mean cortisol plasma level increased slightly although not significantly with both doses. No significant change in the percentage binding of aldosterone to the heat-labile plasma protein-bound fraction of aldosterone was observed. HBF, as estimated by the fractional clearance of indocyanine green, decreased by 18% and 33% with subpressor and pressor doses of angiotensin II, respectively. Calculated secretion rates of aldosterone (and cortisol) increased disproportionately less than the corresponding plasma levels (Fig. 1). Furthermore, significant negative correlations between the MCR of each steroid and the respective values of the fractional clearance of

TABLE 1 Effect of Angiotensin II in Subpressor and Pressor Doses on the Metabolic Clearance Rates (MCR) of Five Steroids, Plasma Level of Aldosterone and Cortisol, Protein-Bound Fraction of Aldosterone, and Hepatic Blood Flow as Estimated by the Fractional Clearance of Indocyanine Green (ICG)

	A II subpressor		A II pressor	
	0 min	60 min	120 min	130 min
MCR (liters/24 hr/m <sup>2</sup> )				
Aldosterone	812.3 ± 34*	622 ± 17†	509 ± 28†	510.1 ± 27†
Cortisol	225.5 ± 11	189.4 ± 10‡	177.2 ± 11†	178 ± 11†
11-Deoxycorticosterone	817.6 ± 51	604 ± 36†	488.9 ± 31†	496.7 ± 42†
Progesterone	1333 ± 122	898.7 ± 86†	766.8 ± 73†	770.5 ± 85†
Corticosterone	678.5 ± 35	582.6 ± 44‡	505.4 ± 34†	480.6 ± 32†
Plasma levels				
Aldosterone (ng/100 ml)	8.7 ± 2.6	27.6 ± 4.2†	34.8 ± 4.3†	37.7 ± 7†
Cortisol (μg/100 ml)	11.3 ± 1.2	14.5 ± 2.6	14.3 ± 2.4	14.4 ± 2.2
Protein-bound fraction of aldosterone (%)	8.4 ± 1.4	9.8 ± 1.5	9.3 ± 1.8	8.8 ± 2.1
Fractional clearance of ICG (min <sup>-1</sup> )	0.199 ± 0.109	0.163 ± 0.08†	0.134 ± 0.075†	

Results are expressed as mean ± SEM.

\* Normal values of our laboratory in 32 control subjects (including the present values): 1,043.8 ± 58 liters/24 hours per m<sup>2</sup> (range, 509–1,810).

†  $P < 0.001$  (significance of difference vs. baseline values).

‡  $P < 0.01$ .

indocyanine green were observed (Table 2). The correlation was slightly less close for the MCR of cortisol ( $r = -0.583$ ) and corticosterone ( $r = -0.655$ ) although still highly significant.

### Discussion

The present findings demonstrate that angiotensin II in subpressor and pressor doses causes a marked, nonselec-

tive decrease of the MCR of aldosterone, cortisol, DOC, corticosterone, and progesterone. This decrease in MCR closely parallels the decrease in HBF, which is produced by the vasoconstrictive action of angiotensin II on the splanchnic vascular bed. The effect of angiotensin II on splanchnic blood flow is well documented from animal studies<sup>10, 16</sup> and in normotensive man with or without liver disease.<sup>9, 17</sup> A decrease in HBF also was found in experimental renal hypertension in the rat<sup>18</sup> and confirmed in our laboratory for hypertensive patients with renal artery stenosis and a renal venous plasma renin ratio (value of the affected side divided by that of the nonaffected side) of more than 2.0.<sup>19</sup> The diminished HBF slows down the inactivation of various physiological and pharmacological agents by the liver. These substances are cleared from the circulation according to the formula  $HC = HBF \times E$ , in which HC denotes hepatic clearance and E, hepatic extraction (arteriovenous difference in concentration divided by arterial concentration of the substance). Provided that the hepatic extraction remains constant and is nearly complete ( $E = 1$ ) (a condition which applies for several steroids<sup>20</sup>) the hepatic clearance depends entirely on the HBF.

Our findings of an angiotensin II-induced decrease in

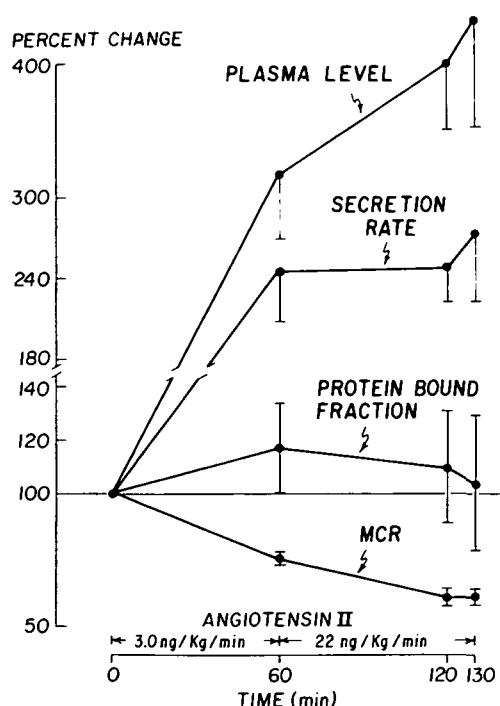


FIGURE 1 Effect of angiotensin II in subpressor and pressor doses on plasma level, calculated secretion rate, protein-bound fraction, and metabolic clearance rate (MCR) of aldosterone. Values are expressed as percentage change of the baseline (=100%).

TABLE 2 Correlation between the Fractional Clearance of Indocyanine green [Hepatic Blood Flow (HBF)] and the Metabolic Clearance Rate (MCR) of the Five Steroids before and during Angiotensin II Infusion

	HBF/MCR	
	Correlation coefficient*	Equation of regression
Progesterone	-0.884	$y = -845x + 393$
Aldosterone	-0.871	$y = -358x + 551$
11-Deoxycorticosterone	-0.854	$y = -421x + 589$
Corticosterone	-0.655	$y = -231x + 207$
Cortisol	-0.583	$y = -577x + 102$

\* All correlations are highly significant ( $P < 0.001$ ).

the MCR of aldosterone are at variance with those of Oelkers et al.,<sup>21</sup> who, however, have measured the biological half-life of aldosterone by single injection before and during angiotensin administration in only one subject. Furthermore, Vecsei et al.<sup>22</sup> measured the MCR of aldosterone in patients with various diseases and did not find any persistent changes in patients with renal artery stenosis for whom, however, no data on plasma renin activity were available. Johnson et al.<sup>23</sup> reported a normal metabolism of aldosterone in dogs with experimental renal hypertension. However, one-kidney hypertension in the dog probably does not correspond entirely to the renovascular hypertensive syndromes observed in the human. This view is supported by the findings of Kaufmann et al.,<sup>24</sup> who found a marked decrease in the MCR of aldosterone in patients with renal artery stenosis, which was partially reversed by corrective surgery.

If one considers the various correlations between the HBF and the MCR of the five steroids it becomes obvious that the MCR of cortisol and corticosterone are slightly less dependent on HBF than are those of progesterone, aldosterone, and DOC. This can be explained by the fact that cortisol and corticosterone are strongly bound to transcortin and that the transcortin-steroid complex is extracted by the liver not at all, or to a very low extent.<sup>20</sup>

It must be emphasized that during an angiotensin II infusion, plasma levels do not reflect the corresponding secretion rates. Because of the decrease in the MCR of all steroids, the respective secretion rates are disproportionately lower than the plasma levels and, by considering the latter only, a marked overestimation of the stimulatory effect would result.

We conclude that angiotensin II in subpressor and pressor doses decreases the MCR of aldosterone, cortisol, DOC, corticosterone and progesterone. This effect seems to be mediated by its vasoconstrictive action on the splanchnic vascular bed. Although hypertension induced by angiotensin II infusion may not be entirely analogous to various forms of renovascular hypertension, the present findings suggest that a decrease in the MCR contributes to the high plasma levels of aldosterone encountered under those conditions. The effect of angiotensin II on plasma steroid concentration is mediated to a variable extent by direct stimulation of the adrenal cortex, by a release of ACTH, and by a decrease in the MCR.

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