Renin suppression by DOC and NaCl in the rat

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Pettinger, William A., Mary Marchelle, and Loraine Augusto. Renin suppression by DOC and NaCl in the rat. Am. J. Physiol. 221(4): 1071-1074. 1971.—Endocrine and dietary changes known to alter plasma renin activity (PRA) were studied in the nonanesthetized rat. Deoxycorticosterone (DOC) suppressed PRA by 90-95%. Ingestion of a low-sodium diet elevated PRA from a mean control value of 120 to 530 ng/100 ml per hr. This elevation was diminished or reversed by increments of sodium in the drinking water. Thus, changes in PRA in the rat are qualitatively similar to those in other species. Sodium pentobarbital and other anesthetics increased PRA to levels similar to anesthesia control values reported by other investigators using these agents in their standard experimental procedure. Thus, normal levels (approximately 100 ng/100 ml per hr) of PRA in the unanesthetized rat appear to be much lower than reported in the anesthetized rat. Either sodium or pentobarbital anesthesia also abolished the depression of PRA induced by administration of DOC and sodium. This artifactual elevation of plasma renin, and thus angiotensin, activity by anesthetic agents can explain: 1) the previously reported failure of DOC to suppress renin activity in the rat (9), and 2) the limited aldosterone secretory response to angiotensin in the anesthetized rat. These results suggest that the nonanesthetized rat is a good subject for physiologic and pharmacologic studies of the renin-angiotensin system.

renin and anesthesia; aldosterone; renin release

Failure to suppress plasma renin activity (PRA) by administration of deoxycorticosterone (DOC) to the rat (9), along with other inconsistencies (3, 12, 16), suggested a quality unique to this species and probably directed investigators to the use of less convenient laboratory animals. However, anesthesia by ether or barbiturate increases plasma renin levels (14) in the rat, an artifact which could qualitatively explain the species inconsistencies in each of the reports listed above. Therefore, physiologic and pharmacologic techniques known to alter renin in other species were used in nonanesthetized rats. The results of these experiments are reported here.

The effect of a relatively low-sodium diet on PRA with various levels of sodium replacement, and of DOC with and without sodium was studied. Also, the previously reported effect of anesthesia (14) was extended to DOC-Na-treated animals.

Methods

Male Charles River rats, weighing 190-250 g, were housed in individual cages. They were exposed to light by an automated system from 6 AM to 6 PM. The rats in a given experiment were killed between 9 and 11 AM. The rats ingested (ad libitum) tap water containing 1 mEq of sodium per liter or saline concentrations as indicated in results. The diet consisted of Purina rat chow (160 mEq Na/kg) or a sodium-deficient diet (50 mEq Na/kg) obtained from General Biochemicals. Pellets containing 25 mg DOC (Mann Research Laboratories) were made in the Pharmacy Research and Development Section at Hoffman-La Roche Inc. The pellets also contained sugar 6X from Merck & Co. (7.6 mg), gelatin (0.5 mg), and magnesium stearate (0.16 mg). Pellets for the control animals were identical but contained inert material instead of the 25 mg of DOC. Two pellets were placed subcutaneously through an incision on the dorsum of the neck of each animal.

Volatile anesthetics were administered by placing the animals in glass jars containing an ether, Metofane, or water (control)-soaked cotton gauze. Sodium pentobarbital (Diabutal), 30 mg/kg, was administered intraperitoneally in the form of a 30 mg/ml saline solution. An equal volume (0.2 ml) of saline was administered to control animals. The anesthetized rats were killed by decapitation at the moment they lost righting reflexes. An equivalent period was allowed for the sham-treated controls.

Aortic blood samples were collected during the first 4 sec after decapitation. The collection tubes contained sodium-EDTA at an approximate final concentration of 3 X 10^{-3} M. The blood was cooled by ice immersion and centrifuged at 4 C at 12,000 X g for 20 min. The plasma was removed and stored at 20 C. Prior to incubation it was thawed and treated with a 5% solution of diisopropylfluorophosphatethe DFP) (v/v, 1/10) in isopropanol and the pH adjusted to 5.9 with 0.5 N HCl. This plasma mixture was incubated at 37 C for 2 hr, immersed in flaked ice, and taken to a cold room where angiotensin I radioimmunoassay was done as described below.

Development of antibodies. The carbodiimide technique of Goodfriend et al. (7) was used to couple angiotensin I to protein. In our experience of immunizing 60 rabbits, bovine gamma globulin was more effective in developing antibodies (85% success) than rabbit serum albumin (15% success) as the protein portion of the antigen. Antibodies were developed in New Zealand white rabbits by fortnightly intracutaneous and toe-pad injections. Blood containing antibodies was obtained by ear artery puncture or by exsanguination from the carotid artery and allowed to clot overnight at 4 C. The serum was heated to 54 C for 30 min and cooled.

The gamma globulin fraction was precipitated with 33% ammonium sulfate and dialyzed for 3 days against 0.1% sodium chloride. This fraction was then taken up to
the original plasma volume and stored frozen in 1 ml aliquots. At the time of assay (see below) this antibody was diluted 1/8,000 in 0.1 M Tris buffer, pH 7.5, which contained lysozyme (300 mg/100 ml).

Iodination of angiotensin I. Angiotensin I (Asp¹-Ileu₅-angiotensin I), obtained from Schwarz BioResearch, was iodinated by the chloramine-T method of Hunter and Greenwood (11) and Boyd et al. (2), with several modifications. Three microcuries of carrier-free Na¹²⁵I (Amersham-Searle) were taken up in 50 μl of 0.5 M NaH₂PO₄ buffer, pH 7.4. As in the technique of Catt and Culross (4), 25 μl of angiotensin I, 400 μl/ml, were taken up into a 25-μl capillary tube connected to a 5-ml syringe. This was followed in the tube by 10 μl of buffer and 40 μl of chloramine-T (Eastman), 2 mg/ml, with a small air bubble between each sample. This mixture was instantaneously injected into the Na¹²⁵I, mixed 5 sec, and the reaction stopped with 0.1 ml of sodium metabisulfite, 1 mg/ml.

Free Na¹²⁵I and some peptide fragments were separated from Na¹²⁵I-angiotensin I by chromatographing the mixture on a 20 x 0.6 cm column of CM-Sephadex, equilibrated in 0.05 M sodium acetate buffer, pH 5.0. Iodinated angiotensin I was eluted with 0.1 M sodium acetate, pH 7.5. The peak consisting of 4 ml of eluate was pooled and diluted in 0.1 M tris buffer, pH 7.5, divided into aliquots and frozen.

Renin assay per se. This step was done entirely in the cold room at 4 C. Triplicate 0.1-ml aliquots of the incubated plasma samples (see above) were placed in siliconized glass tubes containing 2 μl of Na¹²⁵I-labeled angiotensin I. Diluted antisera (4 ml) (see above) was added to each sample. A standard curve was determined with each experiment in an identical manner, with the use of 0.1 ml of nonincubated DFP-treated plasma. Appropriate internal standards of angiotensin I were included with each group of animals in a given experiment.

After overnight incubation at 4 C, the free angiotensin I was precipitated by addition of 0.1 ml of dextran-coated charcoal (6) to the samples and centrifugation at 1,200 X g for 20 min at 4 C. The antibody-bound Na¹²⁵I-labeled angiotensin I was decanted in the supernatant and the charcoal button containing the free Na¹²⁵I-labeled angiotensin I was counted in a Packard Autogamma spectrometer. Quantities of angiotensin I in the unknowns were determined directly from the standard curve. Internal standards were placed in each group of similar samples in order to confirm the correct position of standard curves. Since recoveries were consistently between 89 and 113 %, the values reported here are uncorrected.

RESULTS

Plasma renin activity (PRA) was significantly increased by ether (P < 0.025) and sodium pentobarbital (P < 0.01) anesthesia in normal rats (Fig. 1). PRA appeared to be increased by Metofane anesthesia but this was not statistically significant (P > 0.1).

![FIG. 1. Effect of anesthesia on PRA of normal rats. Sodium pentobarbital, 30 mg/kg, was given ip and ether and Metofane by inhalation. Each value is mean of 5 animals ± se.](http://ajplegacy.physiology.org/ Downloaded from http://aje20335.33.33.on.April.1/)
Subcutaneous implantation of DOC pellets suppressed PRA by 86% \((P < 0.001)\) within 24 hr and 95% \((P < 0.001)\) in 3 days (Fig. 2). This low level of PRA persisted for at least 2 weeks, confirming the prolonged effect and apparently slow release of DOC from the pellets. The presence of sodium chloride (0.9%) in the drinking water appeared to accelerate the DOC-induced suppression of renin, but did not add to the effect of DOC alone after 3 days (Fig. 2).

Ether and pentobarbital anesthesia markedly increased \((P < 0.01)\) the PRA from the DOC-Na-suppressed levels (Fig. 3). The PRA in the pentobarbital-treated rats was unusually high in this experiment and quite variable.

Rats ingesting a relatively low-sodium diet (50 mEq Na/kg) for 5 days had a fourfold higher PRA (Fig. 4) than rats on a normal diet \((P < 0.001)\). The ingestion of 0.01% or 1.0% NaCl in lieu of tap water by these rats restored PRA toward \((0.01 \% \text{ NaCl})\) or to \((1.0 \% \text{ NaCl})\) normal levels. There was a significant difference \((P < 0.01)\) in the PRA on the 1st day after initiating 0.01% and 1.0% sodium chloride in the drinking water. However, this difference became insignificant \((P > 0.1)\) by the 3rd day of saline ingestion.

**DISCUSSION**

Deoxycorticosterone (DOC) markedly lowered PRA in normal rats in the absence of anesthesia. Ingestion of a low-sodium diet elevated PRA. This elevation was diminished or abolished by different sodium concentrations in the drinking water. Thus, changes of PRA in response to DOC and sodium in the rat are qualitatively similar to changes in other species \((13)\). PRA was increased by ether and barbiturate anesthetics in normal rats. This effect of anesthesia also overrode the marked suppression of PRA in DOC-sodium treated rats.

These relatively high levels of renin activity in anesthetized rats would be expected to result in the following: A) apparent absence of renin suppression by DOC, as was reported by Goodwin et al. \((8)\); B) high base-line aldosterone secretion with a relatively small or variable increment in response to angiotensin infusion as reported by Marieb and Mulrow \((12)\), Singer et al. \((16)\), and Cade and Perenich \((3)\); C) a normal increment of aldosterone response to angiotensin in nephrectomized rats, as was reported by Marieb and Mulrow \((12)\); and D) high control levels of PRA in barbiturate or ether-anesthetized rats in contrast to much lower levels in the absence of anesthesia (Tables 1 and 2).

Thus, the effect of anesthesia per se can qualitatively explain each of these abnormalities of the renin-angiotensin-aldosterone systems purportedly unique to the rat. The characteristic responses of the PRA to DOC and to dietary sodium deprivation with and without replacement of NaCl in the drinking water suggests that the unanesthetized rat is a useful subject for physiologic and pharmacologic studies of these systems.

Commercially available low-sodium rat diets, such as the one used here, are not severely deficient in sodium but evidently low enough to increase PRA. The rats ingested approximately 16 mEq Na/kg per day while on the low-sodium diet. In other studies the total food intake has not been affected by changing to the low-sodium diet. The total sodium intake in the rats ingesting the low-sodium diet and 0.01% NaCl as drinking water was approximately 10 mEq Na/kg per day. The effect on PRA (Fig. 4) of this increment in sodium intake is not surprising in view of the well-known responsiveness of human PRA to relatively small changes in sodium intake \((F. C. Bartter: \text{ personal communication})\).

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**TABLE 1. Approximate control levels of PRA reported by investigators using anesthetics**

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**TABLE 2. Approximate control levels of PRA reported by investigators not using anesthetics**

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REFERENCES