Total Exchangeable Sodium in Rats with Mestranol-Induced Hypertension (42887)

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Abstract. **Rats were fed a diet containing mestranol, an orally active estrogen, while control rats were fed the same diet without mestranol. After 6 months of these diets, the rats were weighed, blood pressures were measured, and total exchangeable sodium was determined by injecting ^{24}Na and determining the amount of ^{24}Na in the plasma, the plasma Na concentration, and the residual ^{24}Na in each rat. The 16 mestranol-treated rats were hypertensive (mean arterial pressure 135 ± 3 mm Hg) when compared with the 17 controls (116 ± 3 mm Hg). Total exchangeable sodium in the mestranol-treated rats averaged 39.94 ± 0.49 (SEM) mEq/kg body wt, which was very similar to the value of 39.87 ± 0.63 mEq/kg found in the control rats. Thus, no changes in total exchangeable sodium in mestranol-hypertensive rats were found in these studies.**

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Hypertension develops in some women taking oral contraceptives (1–3), but the mechanisms are not completely understood. We have studied hypertension induced in rats by the long-term administration of a synthetic estrogen (4–6). Estrogens are known to promote sodium retention (7–10), and a relationship exists between sodium and hypertension (11). Therefore, sodium retention may be involved in the etiology of hypertension induced by oral contraceptives in this rat model. The present study was designed to provide information on this possibility by determining whether rats with mestranol-induced hypertension have increases in total exchangeable sodium.

Materials and Methods

A total of 40 female Sprague-Dawley rats (Charles River Laboratories), with initial body weights of 128–156 g, were used in this study. The rats were housed six per cage and were kept at a constant environmental temperature of 27°C; the room was illuminated from 7:00 a.m. to 7:00 p.m. daily by the use of an automatic time switch. Water and food were allowed *ad libitum*.

The rats were fed a commercial rat diet (Rodent Laboratory Chow no. 5001, Ralston Purina) that had been finely powdered. Twenty rats were fed the powdered diet to which mestranol (3-methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-17-ol; Sigma), a synthetic estrogen, had been added in the amount of 112 μg/kg of chow; 20 control rats were fed the powdered chow without mestranol added. The rats received these diets for 6 months, at which time the acute experiment was performed.

Because the acute experiment was performed on conscious rats, beginning 5 days before the acute experiment each rat underwent a training period so that it would remain quietly in a restrainer (4–6); this reduced the rat's anxiety and resulted in more stable blood pressure readings. The restrainer used in these training sessions was the same or was identical to the one used in the experiment. On the first day of training, each rat was placed in the restrainer for 5 min. For the next 2 days of training, each rat received 15 min of training, and on the last 2 training days each rat remained in the restrainer for 30 min.

The day before the acute experiment, food was removed from all rats to obviate variations in body weight and sodium intake resulting from variations in food intake during the final day. Mestranol treatment of the experimental rats was accomplished on this day by the oral injection of 2 ml of water containing 4 μg of mestranol; this dose of mestranol approximated the average daily ingestion of mestranol in the food. Control rats received 2 ml of water orally without mestranol.

On the day of the acute experiment, each rat was weighed to the nearest gram. Each rat then was anesthetized with 2% halothane in nitrous oxide and oxygen, and polyethylene catheters (PE-50 tubing) were implanted surgically in the carotid artery and jugular vein, as described previously (4–6). Each mestranol-

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treated rat then received an oral administration of 2 ml of water containing 4 μg of mestranol, while each control rat received 2 ml of water without mestranol, as on the previous day. Rats then were placed in individual cages and were allowed to recover for 3 hr. At the time of the acute experiment each rat was placed in a restrainer and after being allowed to remain undisturbed for 15 min, the rat's mean arterial pressure was recorded through the carotid catheter for 5 min by use of a pressure transducer (Statham, P23Db) and an oscillographic recorder (Hewlett-Packard, model 7754B).

Total exchangeable sodium was determined in each rat by a modification of the method described by Miller and Wilson (12). Each rat was again anesthetized with halothane. The arterial catheter was knotted, cut, and retracted under the skin. Each rat received an iv injection, through the venous catheter, of 100 μl of water containing approximately 50 μCi of ^{24}Na in a Hamilton syringe, and this was flushed through the catheter with 200 μl of 5% dextrose-water. The venous catheter then was knotted, cut, and retracted beneath the skin. An aliquot of 100 μl of the ^{24}Na solution also was added to a 50-ml volumetric flask, which was diluted to volume with isotonic saline; this formed the ^{24}Na stock standard solution. The ^{24}Na, obtained from the Research Reactor Facility of the University of Missouri in the form of $^{24}Na_2SO_4$, had been neutralized by the addition of HCl and had been appropriately diluted. After being injected with the isotope, the rats were placed in individual metabolic cages and were provided with water but no feed.

At exactly 24 hr after injection of the isotope, each rat was removed from its cage and was anesthetized again with halothane. A blood sample of 1.5 ml was collected in potassium-EDTA by cardiac puncture. Each rat was then killed by an overdose of halothane.

A plasma sample of 500 μl from each rat was placed in a small counting vial. Two 500-μl aliquots of the ^{24}Na stock standard solution were added to separate counting vials to form the ^{24}Na standard solutions. These standards, plus the plasma sample from each rat, were counted in a heavily shielded, specially designed, well-type gamma counter (components from EG & G). The samples were counted for 1 min each; one ^{24}Na standard was counted immediately before and the other standard counted immediately after counting the plasma sample from each rat. The plasma samples were stored in a refrigerator for 1 week and then were analyzed for sodium concentration on a Beckman model E4A electrolyte analyzer system.

The gamma counter, having a well 3 in in diameter and 6-in deep, allowed the direct determination of the total amount of ^{24}Na remaining in each rat. Each dead rat was placed in a plastic bag into a rigid plastic cylinder slightly smaller than the well of the counter. The rat then was maximally compressed to the bottom of the cylinder and was held in this position by a plunger of an appropriate length, which was then taped to the cylinder. The cylinder, containing the entire rat, was then placed in the well of the gamma counter, and each was counted for 1 min. The remaining 49 ml of the ^{24}Na stock standard was added to another cylinder of the same size and was diluted to 250 ml with saline; this formed the ^{24}Na whole-rat standard solution, which was also counted for 1 min immediately after counting each rat.

All counts were corrected for background. When the counts exceeded 35,500 cpm, the counts were also corrected for resolving time. The counts for the whole rats and for the 49-ml ^{24}Na whole-rat standard were also corrected for geometry. Because the plasma samples and the whole rats were each counted at the same time as their appropriate standards, no corrections in the count rates were necessary to allow for isotope decay.

The amount (cpm) of ^{24}Na injected into each rat was calculated by:

$$\text{cpm } ^{24}\text{Na injected} = \text{cpm } ^{24}\text{Na standard solution} \times \frac{100}{}$$

The amount (cpm) of ^{24}Na lost from the rat during the 24-hr equilibration period was calculated by:

$$\text{cpm } ^{24}\text{Na lost} = {}^{24}\text{Na injected} \times (1 - (\text{cpm whole rat}/\text{cpm whole rat standard}))$$

The total exchangeable sodium for each rat was calculated by:

$$\text{Exchangeable Na} = ({}^{24}\text{Na injected} - {}^{24}\text{Na lost}) \times \frac{\text{plasma Na concentration (mEq/liter)}}{2 \times (\text{plasma cpm}/500\ \mu\text{l})}$$

Values for total exchangeable sodium were expressed as mEq of sodium per kg of body weight.

Values for body weight, mean arterial pressure, plasma sodium concentration, and total exchangeable sodium were compared between the mestranol-treated group and the control group of rats by Student's t test for group observations (13). Significance levels were chosen as $P < 0.05$ and $P < 0.01$.

Results

Data were obtained from 16 mestranol-treated rats and from 17 control rats, as 4 rats died, 2 rats lost blood during surgery, and 1 rat did not receive the complete injection of radiosodium. The values for body weight, mean arterial pressure, plasma sodium concentration, and total exchangeable sodium for both the mestranol-treated and control groups of rats are given in Table I. The mestranol-treated rats had significantly ($P < 0.01$) lower body weights and significantly ($P < 0.01$) higher values for mean arterial pressure than did the control

Table I. Values for Mestranol-Treated Rats and Control Rats

	Mestranol-treated rats (n = 16)	Control rats (n = 17)
Body weight (g)	252[a] ± 5	289 ± 6
Mean arterial pressure (mm Hg)	135[b] ± 3	116 ± 3
Plasma sodium concentration (mEq/liter)	140.2 ± 0.5	141.3 ± 0.6
Total exchangeable sodium (mEq/kg body wt)	39.94 ± 0.49	39.87 ± 0.63

Note. Values are means ± SEM.
[a] $P < 0.01$ that the value for the mestranol-treated group is less than for the control group.
[b] $P < 0.01$ that the value for the mestranol-treated group is greater than that for the control group.

rats. Values for plasma sodium concentration were very similar between the two groups. As can be seen in Table I, the total exchangeable sodium values were very similar between the mestranol-treated rats and the control rats.

Discussion

Earlier studies (4–6, 14) have shown that rats given the oral contraceptive Enovid or its component steroids for 6 months will develop hypertension. The estrogenic compound, mestranol, appears to be the substance responsible for this hypertension, as the administration of the progestin component, norethynodrel, without the mestranol failed to produce hypertension, whereas the administration of mestranol alone or in combination with norethynodrel resulted in high blood pressure (4). The dose of mestranol used in this study and in previous studies from this laboratory (4–6) is about three to four times greater, on a per body weight basis, than the dose normally used in women for contraception. Although this is a large dose, it is not immense, and a dose of this magnitude is necessary to produce hypertension in a large percentage of the rats.

This rat model of oral contraceptive hypertension has several similarities to the hypertension seen clinically in women taking oral contraceptives. The hypertension in rats on mestranol is generally mild (4–6, 14), as it is in women taking oral contraceptives and who develop hypertension (2, 3, 15). Also, rats with mestranol-induced hypertension, as well as women on oral contraceptives, have large elevations in plasma renin substrate (2–4, 16–18). Although we have observed no significant increases in plasma renin activity in rats after 6 months on mestranol (4), plasma renin activity in women taking oral contraceptives has been reported as variably increased (2, 3, 16, 17) or unchanged (18). These similarities suggest that the mechanisms acting to produce hypertension in this rat model may be the same as those responsible for the production of hypertension in women taking oral contraceptives.

Naturally occurring estrogens will cause retention of sodium in human subjects (10, 19) and in dogs (7–9). The sodium retaining action of estrogens appears to be independent of their estrogenic activities, as estriol, a weak estrogen, will cause at least as much sodium retention as the stronger estrogen, estradiol (7). Estrogens are known to cause increases in the secretion of adrenal mineralocorticoids (19), and at least a part of their sodium retaining effects probably are due to this; however, additional mechanisms also are responsible for the retention of sodium by estrogens. Dogs given large doses of deoxycorticosterone acetate (DOCA) for several days show extreme sodium retention followed by a return to sodium balance (DOCA escape); the administration of estradiol to DOCA-escaped dogs still receiving large doses of DOCA resulted in the usual pattern of sodium retention seen following the administration of estradiol to normal dogs (7). Also, adrenalectomized dogs that were undermaintained with mineralocorticoid therapy and in negative sodium balance responded to the administration of estradiol with sodium retention and positive sodium balance (8). The demonstration of estrogen receptors in the proximal tubules of the kidneys of rats has suggested that estrogens may promote sodium retention by acting directly on the proximal tubule cells (20).

The effect of estrogens on body fluid volumes is not well understood. Patients on oral contraceptives have been reported to have increased weight gains, which was presumed to the due to fluid retention (21). However, previous studies from this laboratory (5) failed to find any alterations in plasma volume, extracellular volume, or total body water in rats given mestranol of 6 months.

Total exchangeable sodium has been examined in several rat models of hypertension. Rats with hypertension due to injection with DOCA and drinking saline (DOCA-salt rats) were found to have increases in total exchangeable sodium (22, 23). Also, it has generally been observed that one-kidney rats with renal artery stenosis (one kidney, one clip) and hypertension have greater values for total exchangeable sodium than sham-operated rats (24, 25), whereas in hypertensive two-kidney rats with unilateral renal artery stenosis (two kidney, one clip), total exchangeable sodium was not altered (25, 26). In human subjects, increases in total exchangeable sodium have been reported in women receiving ethynyl estradiol, diethylstilbestrol, or conjugated estrogens (27, 28).

The values for total exchangeable sodium per kg body weight for the rats in this study were very similar to those reported by others for normal rats of a similar size (24, 26, 29). The rats receiving mestranol in this study were hypertensive and failed to gain weight at the same rate as the control rats, as was observed in earlier studies (4–6, 14); however, no increases in total exchangeable sodium were observed. This finding is in

keeping with the finding of an earlier study from this laboratory that rats with mestranol-induced hypertension have normal values for extracellular fluid volumes both on a per body weight basis and on a per lean body mass basis (5). Because mestranol-treated rats have less body fat than normal (5), expressing total exchangeable sodium on the basis of lean body mass would not result in higher values for the mestranol-treated rats. Although bone contains large amounts of sodium (30, 31), and estrogens promote the deposition of bone (32–34), the sodium in bone exchanges very slowly (31) and would not be expected to exchange appreciably with the radiosodium during a 24-hr equilibration period.

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