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Acute Sodium-Retaining Effects of Estrogens in Dogs[1] (39914)

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Estrogens are known to produce sodium retention in laboratory animals (1-4) and in humans (5-7). However, most of the studies on the sodium-retaining effects of estrogens have been concerned with the effects of estrogens injected intramuscularly or subcutaneously during daily sodium balance observations. In an earlier study Dignam *et al.* (5) reported an inability to observe any changes in urinary sodium excretion 60-90 min following the intravenous administration of estradiol in humans, and concluded that estradiol does not alter urinary sodium excretion when administered i.v.; it is possible, however, that the sodium-retaining effects of estrogens are slow in onset. The present study examined the acute effects (over a 4-hr period) of the intravenous administration of estradiol or estriol on the urinary excretion rates of sodium and potassium and the clearance of creatinine in adrenalectomized, ovarectomized dogs. Adrenalectomized dogs were used to prevent adrenal steroid hormones from masking the effects of the estrogens on sodium and potassium excretion rates.

Materials and methods. Five female mongrel dogs, ranging from 17.3 to 20.2 kg in weight, were used in these experiments. Each dog was anesthetized with sodium pentobarbital (30 mg/kg body weight), and both adrenal glands and both ovaries were removed by sterile surgical procedures. Prior to surgery, each dog received 100 mg of cortisone orally, 50 mg of cortisone im, and 5 mg of deoxycorticosterone acetate (DOCA) im; following surgery, each dog received 50 mg of cortisone and 5 mg of DOCA im daily for at least a week, and then was maintained on 25 mg of cortisone and 2.5 mg of DOCA per day. A period of at least 3 weeks was allowed for the dogs to recover from surgery before beginning any experiments. Completeness of the adrenalectomy was verified by placing each dog in a metabolic cage, feeding it a diet of 65 mequiv of sodium per day, and stopping all steroid maintenance for 4 days; a negative sodium balance during this time was used as an indication of complete adrenalectomy.

Three separate experiments were performed on each dog, and at least 10 days were allowed between experiments for each animal. The design of the three experiments was identical, except that in one experiment the dog received an acute injection of estradiol, in another experiment estriol was administered, and in the third experiment the dog received the vehicle alone. The order of these three experiments was randomized.

Four days prior to an experiment, each dog was placed on a diet containing 200 mequiv of sodium and 24 mequiv of potassium per day, and steroid maintenance was reduced to 5 mg of cortisone daily with no DOCA administration; this was essentially the same assay preparation used by Liddle *et al.* (8). The last feeding prior to an experiment occurred exactly 10 hr before beginning the experiment. On the morning of an experiment, a catheter of polyethylene tubing (PE 50) was inserted percutaneously into the saphenous vein and was taped in place to be used throughout the experiment for the continuous infusion of creatinine and for the administration of the estrogen or the vehicle. The urinary bladder was catheterized for the collection of urine. All experiments were performed on conscious dogs; during the experiment the dog was lying quietly on the floor of the laboratory,

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restrained only by a rope loosely fastened around the neck. Thirty minutes before the start of the experiment, a priming dose of creatinine (10 ml of a 10.8% solution in water) was administered and a continuous infusion of creatinine (0.12 ml/min of a 10.8% solution in water) was begun. Each experiment consisted of a 1-hr control period, after which the dog received an iv injection of either 650 μg of estradiol, 650 μg of estriol, or vehicle alone. The estrogens were dissolved in 2 ml of ethanol and then diluted to 24 ml with isotonic saline before injection; the vehicle consisted of the ethanol and saline without estrogens. Following the injection, there were four experimental periods of 1 hr each. The urinary bladder was emptied and washed with about 50 ml of distilled water at the start of the control period and at the end of each clearance period. At the midpoint of each period, a 10-ml blood sample was collected for the determination of plasma creatinine concentration and the plasma concentrations of sodium and potassium. After the last clearance period, all catheters were removed and the dog was returned to its cage. Normal steroid maintenance (cortisone, 50 mg, and DOCA, 2.5 mg, per day) and a normal diet were continued until 4 days prior to the next experiment. At least 10 days were allowed between experiments for each dog.

An additional five normal female mongrel dogs with intact adrenals, ranging from 16.6 to 21.4 kg in weight, were placed on a diet containing 200 mequiv of sodium and 24 mequiv of potassium per day for 4 days, and blood samples were obtained for plasma concentrations of sodium and potassium.

Plasma and urine concentrations of creatinine were determined by standard colorimetric procedures, and plasma and urine concentrations of sodium and potassium were measured by flame photometry. The effects of estrogens or vehicle on the urinary excretion rates of sodium and potassium, plasma concentrations of sodium and potassium, and the clearance rates of creatinine were tested statistically by comparing the values for these factors during the control periods with those during each hourly period following the test injection by Student's *t* test for paired observations (9). Statistical comparisons of the plasma concentrations of sodium and potassium between the adrenalectomized, ovarectomized dogs and the dogs with intact adrenals were made by Student's *t* test for group observations (9).

Results. The effects of estradiol on urinary sodium and potassium excretion rates are shown in Figs. 1 and 2. The intravenous injection of 650 μg of estradiol resulted in a progressive decrease in urinary sodium excretion, which declined from an average of 154 ± 7 (SEM) μequiv/min during the control period to 122 ± 19, 103 ± 18, 79 ± 12, and 63 ± 7 μequiv/min during the first through fourth hours, respectively, following the estradiol administration; the changes in sodium excretion were statistically significant ($P < 0.01$) for the third and fourth hours. The urinary excretion of potassium also decreased progressively from an average control value of 22 ± 4 to 10 ± 4 μequiv/min during the fourth hour. The

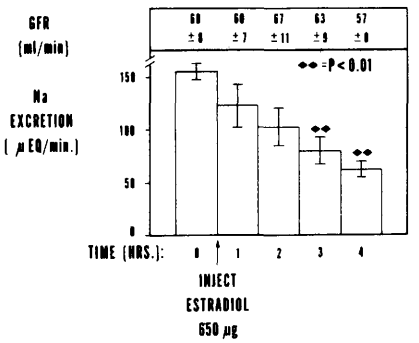

FIG. 1. Effect of estradiol on urinary sodium excretion and glomerular filtration rates (GFR) in five adrenalectomized dogs (means ± SEM).

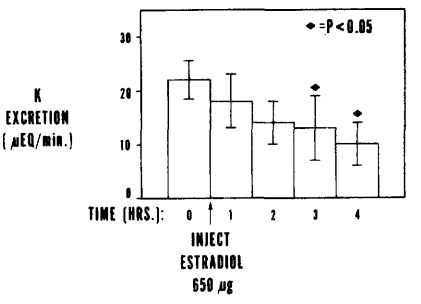

FIG. 2. Effect of estradiol on urinary potassium excretion in five adrenalectomized dogs (means ± SEM).

changes in potassium excretion were also statistically significant ($P < 0.05$) during the third and fourth hours. Creatinine clearance rates, which were used as measurements of glomerular filtration rates (GFRs), did not change during the 4 hr following estradiol administration.

The injection of 650 μg of estriol also resulted in decreases in the urinary excretion rates of sodium and potassium, with no changes in GFRs (Figs. 3 and 4). Urinary sodium excretion decreased from an average value of 139 ± 8 μequiv/min during the control period to 90 ± 12, 75 ± 13, 65 ± 14, and 56 ± 16 μequiv/min during the first through the fourth hours, respectively, following the administration of the estrogen; these changes were statistically significant at each time period. Urinary potassium excretion also decreased from 21 ± 2 μequiv/min during the control period to 9 ± 3 μequiv/min during the fourth hour following estriol injection. The changes in potassium excretion were statistically significant during the second through the fourth hours. Administration of the vehicle alone did not produce changes in the urinary excretion rates of either sodium or potassium, and the GFRs, likewise, were unaltered.

Plasma sodium concentrations in the five adrenalectomized dogs on the high-sodium diet averaged 142.3 ± 0.7 (SEM) mequiv/liter, which was significantly ($P < 0.01$) less than the plasma sodium concentration of 146.6 ± 0.7 mequiv/liter found in the normal dogs on a similar diet. Also, the plasma potassium concentration of 5.3 ± 0.2 mequiv/liter for the adrenalectomized

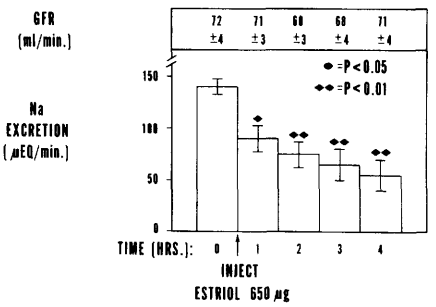

FIG. 3. Effect of estriol on urinary sodium excretion and glomerular filtration rates (GFR) in five adrenalectomized dogs (means ± SEM).

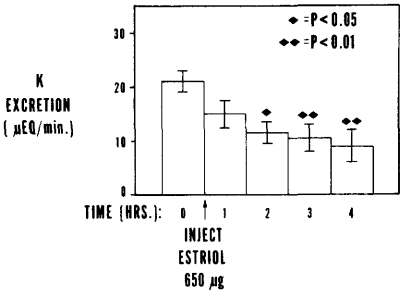

FIG. 4. Effect of estriol on urinary potassium excretion in five adrenalectomized dogs (means ± SEM).

dogs was significantly ($P < 0.01$) higher than the plasma potassium concentration of 4.2 ± 0.2 mequiv/liter in the normal dogs. Following the injection of the vehicle or either estrogen, the plasma sodium concentration was unaltered. After the administration of estradiol, however, the plasma potassium concentration decreased from an average value of 5.4 ± 0.3 to 4.4 mequiv/liter by 3.5 hours ($P < 0.01$); estriol injection resulted in a decline in plasma potassium concentration from an average of 5.2 ± 0.1 mequiv/liter during the control period to 4.5 ± 0.1 mequiv/liter after 3.5 hr ($P < 0.05$). Following the injection of the vehicle alone, however, a significant decline in plasma potassium concentration was also observed; plasma concentration of potassium decreased from an average of 5.5 ± 0.3 mequiv/liter during the control period to 4.7 ± 0.4 mequiv/liter by 3.5 hr ($P < 0.01$). Comparing the plasma potassium concentrations at each period for all three experiments by an analysis of variance (9) revealed no differences among the three experimental groups for any time period; thus, the changes in plasma potassium concentration with time were the same following vehicle administration as they were following estrogen administration.

Discussion. There are several reports that the chronic administration of estrogens will result in a decreased urinary excretion of sodium. Thorn and Engel (4) observed that the injection of 5 mg of estradiol subcutaneously into dogs produced a marked decrease in urinary sodium excretion for several days. Similar results were seen by Dance *et al.* (1) following the subcutaneous injection of stilbestrol in dogs. Earlier work

from this laboratory (2) demonstrated that the im injection of estradiol in doses of 100 μg, 250 μg, or 1 mg per day for 7 days resulted in decreased urinary excretion of sodium, and this effect was most pronounced at the higher doses; no variations in urinary potassium excretion were seen. The injection of estriol in doses of 100 or 250 μg per day for 7 days also resulted in sodium retention (2). Estrogen administration to human subjects has also been reported to result in sodium retention (5–7).

A possible mechanism whereby estrogens could decrease the urinary excretion of sodium would be by decreasing the glomerular filtration rate (GFR). However, investigations by several groups have failed to demonstrate a lowering of the GFR in response to estrogens in humans (5, 10) or dogs (3, 11–13). In the present study, the GFR, as determined by creatinine clearance, was unaltered following the injection of estradiol or estriol. Thus, estrogen-induced sodium retention is apparently not mediated by decreases in GFR.

It is well established that the administration of estrogens will promote increased aldosterone secretion (14–17), and the sodium retention which occurs following the administration of estrogens to intact animals may be due in part to this adrenal steroid. However, that estrogens also produce sodium retention by additional mechanisms was firmly established by earlier studies from this laboratory, which revealed that the administration of estradiol to adrenalectomized dogs still resulted in a pronounced retention of sodium (3); this observation was further verified by the present experiments. Furthermore, in earlier studies, the administration of estradiol to dogs that were receiving large doses of DOCA and that exhibited "sodium escape" still resulted in the usual degree of sodium retention (2). Because DOCA presumably saturated nearly all of the renal tubular receptor sites for adrenal mineralocorticoids, these experiments indicated that estradiol was probably acting at different receptor sites in the kidney than those for the adrenal steroids. Further evidence for this idea was provided by DeVries *et al.* (18); using kidney slices from adrenalectomized male rats, these researchers found that the addition of large amounts of unlabeled aldosterone to the incubation media had no effect on the binding of estradiol.

The effects of estrogens on potassium balance and urinary potassium excretion are of interest, although the mechanisms involved are probably complex and cannot be explained adequately at the present time. In previous experiments (2), the administration of estradiol or estriol to normal, intact dogs for 7 days did not alter the potassium balance. However, when adrenalectomized dogs that were inadequately maintained on adrenal steroids were treated with estradiol for 8 days, a pronounced decrease in urinary potassium excretion was observed (3); a significant fall in plasma potassium concentration accompanied the potassium retention. These results suggested that the retained potassium probably was passing intracellularly. Also, the decreased urinary potassium excretion resulting from estradiol administration might have been related to the decrease in plasma potassium concentration, which could result in a decreased filtered load of potassium. In the present study, a substantial fall in urinary potassium excretion was observed following the administration of estradiol or estriol, but not following the administration of the vehicle alone; however, the plasma potassium concentration decreased to the same extent following injection of either the estrogens or the vehicle alone. Thus, the decline in plasma potassium concentration in the present study was not due to the action of the estrogens, whereas the decrease in urinary potassium was due to the estrogens. The observation that estriol administration to adrenalectomized dogs produced potassium retention represented a new finding, and it is of interest that, although estradiol and estriol have vastly different estrogenic activities, these two estrogens produced approximately the same degree of potassium retention.

The present study revealed that, following the intravenous administration of estrogens, a period of 2–4 hr was required before pronounced decreases in the urinary excretion rates of sodium and potassium occurred. These observations offer a possible

explanation for the failure of Dignam *et al.* (5) to demonstrate an effect of intravenously injected estradiol on the urinary excretion of electrolytes in human subjects; these researchers followed the urinary electrolyte excretion rates for only 60–90 min after estradiol injection. Adrenal mineralocorticoids also require 2–3 hr to produce maximum effects on urinary sodium and potassium excretion rates (8). In comparing the time courses of the urinary excretion rates of sodium and potassium following estrogen or adrenal steroid administration, they are somewhat similar, except that the renal response to estrogens may be more prolonged than that of the adrenal steroids; the effects of deoxycorticosterone and aldosterone on urinary sodium and potassium excretion often reach a maximum by the third hour (8), whereas the effects of estrogens on urinary sodium and potassium excretion are most pronounced during the fourth hour and may not yet have reached their peak effect at this time. Another difference, of course, is that the adrenal steroids stimulate a loss of potassium in the urine, whereas potassium retention occurred following estrogen administration in the present study. The time course of the retention of both sodium and potassium following the administration of estriol was approximately the same as that following the administration of estradiol. In earlier experiments from this laboratory (2), the administration of these two estrogens im to dogs for several days at two dose levels resulted in almost the same degree of sodium retention; the observation in the present study that the degree of sodium retention was about the same following the iv administration of either estradiol or estriol was in agreement with the findings of the earlier study. As estriol has considerably less estrogenic activity than does estradiol, there appears to be no relationship between the estrogenic activity of an estrogen and its sodium-retaining effects.

Summary. Five conscious adrenalectomized dogs on a high-sodium diet received iv injections of 650 μg of estradiol, 650 μg of estriol, or vehicle alone. Every animal received each of the three injections on separate days. One hour prior to and 4 hr following the injection, the urinary excretion rates of Na and K, creatinine clearances, and plasma Na and K concentrations were determined. After administration of estradiol, the urinary Na excretion decreased from 154 ± 7 (SEM) to 63 ± 7 μequiv/min ($P < 0.01$); urinary K excretion also declined from 22 ± 4 to 10 ± 4 μequiv/min ($P < 0.05$). Following the injection of estriol, urinary Na excretion fell from 139 ± 8 to 56 ± 16 μequiv/min ($P < 0.01$), while urinary K excretion decreased from 21 ± 2 to 9 ± 3 μequiv/min ($P < 0.01$). Administration of the vehicle alone did not alter the rate of urinary excretion of Na or K. No detectable changes in creatinine clearance occurred following administration of estrogens or the vehicle. Plasma Na concentration remained unaltered, but plasma K declined slightly in all experiments. These studies demonstrated that estrogens administered iv require 2–4 hr for pronounced effects on urinary Na and K excretion, and that the acute Na-retaining effects of estrogens are not mediated by adrenal steroids or by decreases in glomerular filtration rate.

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