Renal Na excretion in dehydrated and rehydrated adrenalectomized sheep maintained with aldosterone

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IN ADDITION TO THIRST AND greater secretion of vasopressin, increased renal excretion of Na is a physiological response to water deprivation in a number of species (2, 7, 11, 14, 17, 18, 25, 28, 30). If such a dehydration-induced natriuresis is prevented in water-deprived animals, as can occur with certain cerebral lesions, severe hypernatremia ensues (1, 15, 23). Consequently, it has been proposed that the dehydration-induced natriuresis is an important mechanism for buffering increases in plasma Na concentration and osmolality occurring in dehydrated animals (13, 14, 23).

In addition to the aforementioned effects of cerebral lesions preventing dehydration-induced natriuresis (1, 15, 24), experimentally induced reduction of the Na concentration of the cerebrospinal fluid also prevents the increase of Na excretion in dehydrated animals (9, 13, 16, 22), which is further evidence of a role for the brain in this response. A humoral signal to the kidney mediating the natriuresis of dehydration seems likely because it occurs normally in sheep with denervated kidneys (20). Possible humoral mediators that can be excluded by experimental evidence as the causative agent include vasopressin, atrial natriuretic peptide, and the renin-angiotensin system (8, 16, 18, 21, 28). In Na-restricted dogs, blood levels of aldosterone fall when they are water restricted (17), and it has been proposed by some (17, 24), but not all (18, 28) investigators, that reduction in blood aldosterone levels during dehydration may be a major factor in the mechanism of dehydration-induced natriuresis (17, 24). It has also been suggested that the pronounced renal Na retention that occurs when water-deprived animals are rehydrated (3, 14, 18, 28) is caused by the increased plasma aldosterone levels that result (28).

The aim of this study was to test the hypotheses that reduction of aldosterone secretion is the major factor causing dehydration-induced natriuresis and increased aldosterone secretion is the major cause of the marked renal Na retention that follows rehydration. This has been done by 1) measuring the effects of water deprivation and also subsequent rehydration on blood aldosterone levels in intact sheep and by 2) studying renal Na excretion in dehydrated and rehydrated adrenalectomized (ADX) sheep maintained with constant rate infusion of aldosterone and cortisol.

METHODS

Animals

General. Experiments were performed in 17 adult Merino crossbreed ewes of 31–54 kg body wt. All surgical and experimental procedures were approved by the Animal Experimentation Ethics Committee of the Howard Florey Institute, which adheres to the Australian National Health and Medical Research Council’s code of practice for the care and use of animals for scientific purposes. Sheep were housed in individual metabolism cages, fed 0.8 kg of oaten-lucerne chaff daily at 1200, and provided with water at all times except during experimental periods of water deprivation.

Surgical preparation. All sheep were surgically prepared with the carotid arteries enclosed in skin loops in the neck to allow easy withdrawal of arterial blood samples and measurement of arterial pressure, and the ovaries were removed to prevent influences of the estrous cycle on Na and water metabolism. In six sheep, a second operation was performed...
in which both adrenal glands were extirpated several weeks before experimentation (4). After this surgery, these ADX sheep were maintained by intravenous infusion of adrenal steroids. The rates of infusion into a jugular vein via a polyethylene cannula were constant, based on the secretion rates of cortisol and aldosterone in normal, Na-replete sheep, and were 125 µg/h of cortisol and 2.5–3 µg/h of aldosterone infused in isotonic saline solution. Several days before experiments, the exact infusion rate in the range of 2.5–3 µg/h of aldosterone was determined for each sheep as that which maintained plasma Na and K concentrations at preadrenalectomy levels. All surgery was performed while sheep were under the influence of general anesthesia induced by intravenous thiopentone sodium (15 mg/kg) and maintained by inhalation of halothane/oxygen gas.

Experimental Protocols

Experiment 1: measurement of plasma aldosterone concentration during water deprivation for 41 h and subsequent rehydration in intact sheep. On day 1, six normal sheep were weighed, and a blood sample was obtained from the carotid artery. At 1630, water was withdrawn from the animals’ cages for 41 h. Another blood sample was obtained 24 h after the start of the period of water deprivation. After ~39 h, the sheep were weighed and a catheter was inserted into the bladder for urine collections (see experiment 3), and another blood sample was obtained at 41 h. These sheep were then provided with water to drink at room temperature, and blood samples were obtained at 2, 4, 6, and 24 h after rehydration. Plasma aldosterone concentration and electrolyte levels were measured on all samples. As a control for the rehydration period, the same sheep were subjected to the same protocol, except that the period of water deprivation was extended to 65 h. Blood samples were obtained at times corresponding to those obtained in the initial experiment.

Experiment 2: water deprivation for 19 h. The experimental protocol was designed to measure changes in urinary Na excretion during a control day (day 1) and during 19 h of water deprivation (day 2). This was performed on four intact sheep and five of the six steroid-maintained ADX sheep (ADX-constant steroid sheep). On day 1, a bladder catheter was inserted at 0900–1000, and hourly urine collections were commenced at 1100 and, except for a 2-h collection taken between 1800 and 2000, continued until 2200. A single overnight collection between 2200 on day 1 and 0600 on day 2 was made. Then hourly collections continued and followed the same pattern through to 0700 on day 3. Arterial blood samples (10 ml) were obtained at 1130, 1230, 1630, and 2030 on the first 2 days and from 0630–1130 on day 3. Sheep were fed 800 g of chaff at 1200 each day, and food and water intakes were measured each hour. Water was withdrawn from the animals at 1200 on day 2, and it was returned to their cages at 0700 on day 3, which amounted to a period of 19-h water deprivation in all sheep. The volume, [Na], [K], and osmolality of each urine collection were measured, and the plasma aldosterone levels were determined. In addition to the repeated measures analysis of variance, single factor design, followed by Dunnett’s multiple range test to compare the initial value with the final value of the repeated measure, and subsequent multiple comparison tests (Newman-Keuls test) were used to compare electrolyte excretion, plasma osmolality, and ion concentrations of ADX-constant steroid sheep with those of intact sheep during periods of dehydration and rehydration. Repeated-measures analysis of variance, single factor design, followed by Dunnett’s multiple range test to compare the initial value with the following values of the repeated measure were used to analyze results in animals rehydrated after 41 h of water deprivation.

RESULTS

Experiment 1: effect of water deprivation and subsequent rehydration on plasma aldosterone concentrations in normal sheep. Daily plasma aldosterone levels did not change significantly after either 24 or 41 h of water deprivation in six intact sheep (Fig. 1). These six sheep lost 3.7 ± 0.2 kg body wt during these 41 h, and the plasma Na concentrations and osmolality increased significantly during this time (Fig. 1).

On rehydration, the sheep drank 3.12 ± 0.4 l of water, and plasma aldosterone levels gradually increased so that by 6 h after rehydration, this increase had reached significance at the P < 0.05 level (Fig. 1). The plasma aldosterone levels remained elevated 24 h after rehydration. No further measurements of aldosterone concentration were made. During this time, plasma Na concentration and osmolality fell significantly so that within 2–4 h of rehydration, they had returned to their predehydration level and by 6 h were significantly less than that value. If the animals were not rehydrated until the following day, no increase in plasma...
aldosterone occurred at 41–47 h of water deprivation, and plasma Na concentration and osmolality remained elevated (Fig. 1). Body weight loss in these sheep was $3.8 \pm 0.4$ kg.

Experiment 2: effect of water deprivation on Na and K excretion in intact sheep or ADX sheep maintained on constant steroid infusion. The results are shown in Fig. 2. Both intact and ADX-constant steroid sheep showed similar patterns of Na and K excretion, and there was no significant difference between the two groups of sheep over the course of the experiment. On day 1 when water was available to drink, renal Na excretion increased rapidly 3–6 h ($P < 0.01$) after feeding had commenced, reached a maximum, then gradually subsided to prefeeding levels in ADX-constant steroid sheep. Changes in renal Na excretion showed a similar trend in intact sheep, although the postprandial increase in Na excretion did not reach statistical significance. It then declined during the remainder of the 24 h. On day 2 when water was unavailable to drink, a similar pattern of urinary Na excretion was observed in both the ADX-constant steroid sheep and intact group of sheep. The magnitude of the postprandial natriuresis during the first 9 h after feeding tended to be greater than on day 1, and the rate of Na excretion remained at a significantly ($P < 0.01$) elevated level for the subsequent 10 h of water deprivation in both groups. Consequently, in both the intact and ADX-constant steroid sheep, the total amount of Na excreted in urine during the 19 h of water deprivation was significantly more than that excreted during the corresponding period when water was available (Fig. 3). The total amount of K excreted did not change significantly with water deprivation in either intact or ADX-constant steroid sheep (Fig. 3). A similar reduction of body weight occurred in ADX-constant steroid sheep (2.6 ± 0.4 kg) and intact sheep (2.9 ± 0.4 kg, NS) over the 19 h of water deprivation. Both groups of sheep ate all of their food ration (800 g) on day 1 as did the intact sheep during the 19 h of fluid deprivation. The ADX-constant steroid sheep group ate 765 ± 25 g of chaff during this period of water deprivation, which was not significantly different from that of the normal sheep.

Plasma Na and K concentration and osmolality increased with feeding and then gradually fell to prefeeding levels in both intact and ADX-constant steroid sheep (Fig. 4). Plasma protein levels increased with feeding, but this effect was more transient. Water deprivation resulted in greater increases in these variables, but values for intact and ADX-constant steroid sheep did not differ significantly (Fig. 4).

Experiment 3: effect of rehydration after 41-h water deprivation on Na and K excretion rates in intact and ADX sheep maintained with constant steroid infusion. Over a 41-h period of water deprivation, ADX-constant steroid sheep lost 2.58 ± 0.43 kg and drank 3.07 ± 0.41 l of water when they were rehydrated. Those values were not significantly different from intact sheep which lost 3.41 ± 0.19 kg and drank 3.41 ± 0.36 l during the period of rehydration. Both groups had similar intakes of food during the 41 h of fluid deprivation. A large reduction in renal Na excretion was observed after rehydration in both ADX-constant steroid sheep and intact sheep, although the Na retention occurred significantly earlier in the group of ADX-constant steroid
sheep (Fig. 5). In the 10 intact sheep that were not rehydrated after 41 h of water deprivation, no significant reduction in renal Na excretion was observed in the subsequent 6 h, and the Na excretion rate at the end of this period of observation was considerably greater than in either group of rehydrated sheep at the corresponding time (Fig. 5). These sheep lost 3.59 ± 0.28 kg during the initial 41 h of water deprivation.

Renal K excretion fell similarly in both intact and ADX-constant steroid sheep during the 6 h of rehydration (Fig. 5). Renal K excretion changed little during the first 5 h of observation in the group of intact sheep that were not rehydrated at 41 h of water deprivation. By 3 h after rehydration, the plasma [Na] and osmolality had fallen back to the levels seen before the period of water deprivation in both intact and ADX-constant steroid sheep (Fig. 6). Plasma aldosterone concentration was measured in four of the ADX sheep maintained by an intravenous infusion of aldosterone and cortisol, and it did not fall during the 41 h of water deprivation. It was 80 ± 10 pmol/l before depriving animals of water, 113 ± 11 pmol/l after 24 h of water deprivation, and 135 ± 9 pmol/l after dehydration for 41 h. The value for plasma aldosterone was 109 ± 14 pmol/l at 3 h after rehydration in the ADX-constant steroid sheep that had been deprived of water for 41 h. Apart from the increase (P < 0.05) occurring at 41 h of dehydration (compared with the initial water-replete level), these values did not change significantly with dehydration or rehydration, yet a very large reduction in Na excretion was observed with rehydration (Fig. 5). Plasma aldosterone concentrations were measured in six of the rehydrated intact sheep in experiment 1 and gradually increased during the postrehydration period as shown in Fig. 1. This did not occur in the six sheep in which the period of water deprivation was extended past 41 h (Fig. 1).

**DISCUSSION**

The lack of a significant change in plasma aldosterone levels after 24 or 41 h of water deprivation shows that it is unlikely that changes in aldosterone secretion have a role in dehydration-induced natriuresis in sheep. This conclusion is reinforced by our observation that ADX sheep maintained on a constant infusion of aldosterone and cortisol still showed a marked increase in renal Na excretion in response to dehydration. Such animals also showed a pronounced natriuresis 3–6 h after rapid feeding. Although it has been suggested previously that reduced aldosterone secretion is the reason that animals shed Na after feeding (22) or when they become dehydrated (17, 24), the present results in sheep indicate that both natriuretic responses are largely independent of changes in aldosterone secretion. This conclusion was also made by Thrasher and...
colleagues (18, 28) from the results of their studies in water-deprived dogs. In addition, this result should rule out other adrenal factors, e.g., ouabainlike factors from the adrenal glands (6) as possible mediators of dehydration-induced natriuresis. It has been suggested that increased plasma aldosterone concentration is the cause of the pronounced reduction of renal Na excretion that occurs with rehydration (28). The significant increase in plasma aldosterone levels that we observed in sheep after rehydration (Fig. 1) is consistent with this proposal. However, the large reduction in renal Na excretion we observed in the ADX-constant steroid sheep after rehydration shows that this effect is largely independent of altered aldosterone secretion.

The similar changes in plasma osmolality and Na and K concentrations which occurred in intact and...
Thus several studies in water-deprived sheep show that their increased Na excretion is centrally regulated and mediated by a hormonal mechanism that is largely independent of the renin-angiotensin-aldosterone system, atrial natriuretic peptide, or vasopressin secretion. Studies in the rat show that oxytocin may play an important role in dehydration-induced natriuresis in this species (9). This is unlikely to be the case in ruminant species because little change occurs in plasma aldosterone levels during dehydration. Measurement of plasma aldosterone in the steroid-maintained ADX sheep, over the course of 41 h of water deprivation followed by rehydration, confirmed that plasma aldosterone levels do not fall during dehydration or increase with rehydration in these sheep. The tendency of plasma aldosterone levels to increase during the period of water deprivation may be related to the gradual reduction of extracellular fluid volume that will occur with dehydration.

Previous studies of water-deprived sheep show that changes in renal Na and K excretion that occur with dehydration and rehydration, respectively, are not changed by renal denervation (20), captopril treatment (16), or hypophysectomy (21), nor can these changes in electrolyte excretion be accounted for by the circulating levels of vasopressin or atrial natriuretic peptide or by the arterial pressure (8, 16, 21). However, ablation of cerebral tissue in the region of the lamina terminalis prevents dehydration-induced natriuresis (1, 15, 23).
oxygen levels during dehydration or rehydration in sheep (24) or goats (24). In addition, hypophysectomy does not abolish the natriuresis of dehydration in sheep (21), although it may do so in dehydrated Na-restricted dogs (17).

Despite hypovolemia and increased plasma renin and angiotensin II levels occurring in dehydrated rats, dogs, and sheep (3, 18, 28, 30), plasma aldosterone levels either do not change or even fall with water deprivation (3, 18, 28, 30; Fig. 1). These data suggest that a regulatory factor (either plasma [Na], osmolality, or an unspecified humoral agent) is preventing the circulating aldosterone levels from increasing in response to the elevated angiotensin II levels that would have occurred in dehydrated animals. This seems to be an appropriate response because if the plasma aldosterone level increased with dehydration, this could have the effect of opposing natriuretic influences on the kidney, making them less effective. Dehydration-induced natriuresis provides a mechanism for buffering the increases in plasma [Na] and osmolality (13–17), and this would be compromised if increased mineralocorticoid action in the kidney occurred in dehydrated sheep.

The large reduction in renal Na excretion that ensued with rehydration after 41 h of water deprivation, which has been observed previously (3, 14, 18), was quite pronounced in the bilaterally ADX sheep maintained with constant steroid infusion. This shows that factors independent of or additional to aldosterone action mediate this effect. A possible explanation is that when rehydration occurs, withdrawal of a dehydration-induced natriuretic mechanism allows the intrinsic Na-retaining effect of aldosterone on the kidney to become more evident. The plasma aldosterone concentrations measured in the ADX sheep infused with aldosterone and cortisol were greater at the time of rehydration than those of intact sheep, and this may explain why the rehydration-induced reduction in renal Na excretion in ADX-constant steroid sheep was more rapid than in intact animals.

With rehydration in normal animals, both plasma renin concentration and blood aldosterone level increase (although slowly) (3, 18, 28), which is appropriate considering that dehydrated animals incur deficits in bodily Na balance (as a result of the dehydration-induced natriuresis) during the period of water deprivation. Thus in addition to the possibility of a natriuretic mechanism being extinguished, increased aldosterone secretion probably plays a role in the Na retention that may persist for some days after rehydration until neutral Na balance is again attained.

A pronounced natriuresis occurs in sheep 3–6 h after the consumption of dry food (16, 22), and it has been asserted that this postprandial natriuresis is the result of plasma aldosterone levels falling (22). In the present experiments, this postprandial natriuresis was observed in normally hydrated or water-deprived, ADX sheep receiving a constant intravenous infusion of aldosterone. This effect also tended to occur in the intact sheep, although it did not reach significance here as it did in our earlier study, which showed that a significant postprandial natriuresis occurs in intact sheep (16), and of similar magnitude to that of ADX-constant steroid sheep. Thus it is unlikely that reduced blood levels of aldosterone are the cause of the increased renal Na excretion that occurs 3–6 h after feeding in sheep. Our previous study showing that experimentally reducing the Na concentration of ventricular cerebrospinal fluid prevents postprandial natriuresis suggests that a central mechanism regulates loss of Na by the kidney after feeding (16).

Perspectives

The near normal regulation of renal Na excretion and plasma electrolyte concentrations in dehydrated and rehydrated ADX sheep maintained on constant infusion of aldosterone and cortisol shows that factors other than the blood levels of aldosterone play important roles in this regulation. Our earlier studies in sheep (13–16) suggest that the brain exerts a major influence on the regulation of renal Na excretion during periods of dehydration and rehydration and that the effector mechanism is hormonal. However, the present results show that changed aldosterone secretion is not the main hormonal mediator of this regulation.

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