

Parallel Effects of DOCA on Salt Appetite, Thirst, and Blood Pressure in Sheep^{1,2} (42705)

M. N. HAMLIN, R. C. WEBB, W. D. LING, AND D. F. BOHR

Department of Physiology, The University of Michigan, Ann Arbor, Michigan 48109

Abstract. Salt appetite was quantified in sheep by measuring the relative amounts of high-salt (266 meq/kg) and low-salt (6 meq/kg) pelleted alfalfa that they ate. Given a choice of these two foods, normal sheep ate twice as much low-salt as high-salt pellets. Following DOCA administration the sheep rapidly developed an increased salt appetite, and after 10 days they ate approximately three times as much high-salt as low-salt pellets. Their choice rapidly reverted to control values after the end of the DOCA treatment. The changes in salt appetite were accompanied by changes in thirst and mean arterial pressure. We hypothesize that these effects of DOCA reflect changes that parallel those this mineralocorticoid causes in the hypothalamic regulatory centers for salt appetite, thirst, and blood pressure. © 1988 Society for Experimental Biology and Medicine.

Mineralocorticoids have major effects on blood pressure, thirst, and possibly salt appetite. These physiological variables are all influenced by central regulatory processes. In 1939 Kuhlman *et al.* (1) observed that administering 200 mg of desoxycorticosterone acetate (DOCA) per day to dogs produced significant increases in blood pressure. Since then, similar responses have been reported in rats (2-5), pigs (6-9), and humans (10). In all species, the increase in arterial pressure is accompanied by an increase in thirst. We have recently observed (11) that DOCA administration also produces these two changes in sheep.

In all species, changes in blood pressure and thirst produced by mineralocorticoid excess can be reversed by a low-sodium diet. In view of this sodium dependence, it is interesting that at least in rats DOCA administration produces an increase in salt appetite (12, 13).

The current study was undertaken to determine whether this change in salt appetite also occurs in sheep. If so, this would represent another centrally regulated variable that is altered by mineralocorticoid excess, supporting the hypothesis that the brain is an important site of action of DOCA.

Materials and Methods. *Animals.* Adult sheep of the Merino strain were used in this study. They weighed between 40 and 50 kg. Those used for the appetite study were housed in individual 3 ft 6 in. × 5 ft pens in a barn (Michigan Reproductive Endocrinological Farm Facility). Those used in the blood pressure, plasma electrolyte, and fluid intake study were housed in similar individual pens near our laboratory.

Water and food. All sheep were given water *ad libitum*. Throughout the entire protocol the drinking water contained 0.1% NaCl and 0.25% KCl. The NaCl was added because in our previous study (11) it facilitated the development of the elevated arterial pressure. The KCl was added to reduce the severity of the hypokalemia developed by sheep in response to DOCA administration. Each morning at 9:00 AM the volume of water remaining in each sheep's pan was measured, and this was subtracted from the starting volume in order to determine how much the sheep had drunk.

Each sheep had continuous access to two foods in separate pans. Both foods were pelleted alfalfa. One pan contained alfalfa to which salt had been added (see below), and the other contained unadulterated alfalfa pellets. Thus the sheep had access to two foods that were identical in composition with the exception of their NaCl content.

The high-sodium alfalfa was prepared by placing 2 kg of pelleted alfalfa in a large rectangular pan, spreading it out so that the food covered the pan uniformly to a depth of

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one or two pellets. A water atomizer was then used to spray 200 ml of a 3.0 M NaCl solution onto the alfalfa. A total of 600 meq sodium was thus applied to the 2.0-kg sample. Every effort was made to spray the alfalfa uniformly. After 100 ml of the NaCl solution had been sprayed on the alfalfa, the pan was shaken vigorously so as to expose the unsprayed surfaces of the pellets. The final 100 ml of NaCl were then sprayed on the alfalfa. The food was allowed to dry overnight. A food assay was carried out on four randomly selected samples of the high-sodium alfalfa to ascertain how much sodium had actually been added and how uniformly it was distributed. These assays were carried out at weekly intervals. The high-salt diet contained an average of 266 meq sodium/kg, never varying more than 10 meq/kg. The low-sodium alfalfa was sprayed using exactly the same procedure detailed for the high-sodium alfalfa, with the substitution of 200 ml of deionized water for the NaCl solution. Analysis showed that it contained 6 meq sodium/kg, which agreed with an analysis given to us by the supplier, Ohio Blenders, Inc.

Each morning at 9:00 AM the amount of food remaining in each pan was weighed to determine how much each sheep had eaten. The positions of the pans containing high-salt and low-salt pellets were alternated daily. The ratio of high-sodium alfalfa eaten to that of the low-sodium alfalfa was calculated for 5-day intervals for each sheep. Designated by the symbol " R_f ," this ratio was used as a measure of salt appetite.

Surgical instrumentation. The sheep were preanesthetized with thiamylal (Surital, Parke-Davis), followed by anesthesia with halothane. The left carotid artery and jugular vein were exposed and implanted with non-occluding catheters. The carotid catheter was advanced until its end was in the descending thoracic aorta, and the jugular catheter was advanced so that its end was in the region of the right atrium. Both catheters were tunneled under the skin to the back and exteriorized on the right side over the lower rib cage. The catheters were used to monitor pressure and to collect blood samples. The sheep wore a protective jacket over their catheters.

Blood pressure measurements. Each morning the sheep were restrained by placement in a sling from which their feet just reached the floor. Catheters were connected via saline-filled tubes to Gould-Statham pressure transducers recording on a Grass polygraph. Mean arterial pressure, systolic and diastolic pressures, heart rate, and central venous pressure were recorded daily between 9:00 and 11:00 AM. Collecting these data took no longer than 20 min per sheep.

Plasma electrolytes. Arterial blood for electrolyte determination was collected into 5-ml plastic tubes prepared with lithium heparin. It was spun down immediately and the plasma was stored at 4°C until assayed with a flame photometer. Daily determinations were made from 5 days before DOCA administration through 5 days after. Additional samples were taken on the 7th and 10th post-implant days, and every 5th day thereafter.

DOCA treatment. For all of the sheep the protocol for this study comprised three phases: (1) a 10-day control period; (2) a 30-day DOCA treatment period; and (3) a 10-day recovery period. The DOCA treatment consisted of a single subcutaneous implantation on Day zero of silicon strips impregnated with DOCA, 100 mg/kg sheep wt. From studies in pigs (7) we have observed that this dose and route of DOCA administration gives an initial peak plasma concentration that lasts for 1 or 2 days, then falls off gradually to plateau at a level greater than 10 times the normal desoxycorticosterone level for at least 60 days. In all of the sheep, treatment was stopped by surgical removal of the DOCA-impregnated strips.

Data and statistical analysis. Unless stated otherwise, all data are presented as the mean \pm the standard error of the mean (SEM). Statistical comparisons between groups were performed by Student's *t* test. A *P* value less than 0.05 was considered statistically significant.

Results. In each of the six sheep used in the salt appetite study, the R_f value—used as a measure of this appetite—rose in response to DOCA treatment. The average R_f rose from a preimplant value of 0.46 to 1.69 during the first 5-day interval after DOCA implant (Fig. 1C). The R_f continued to rise dur-

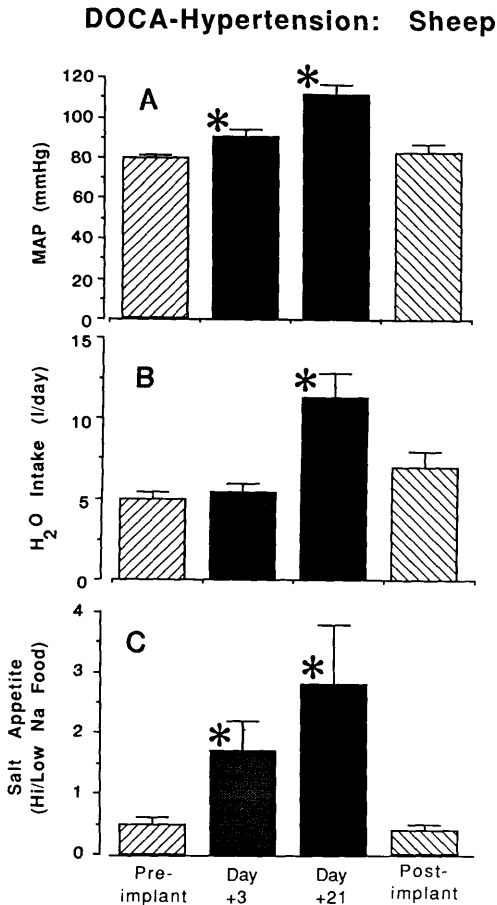


FIG. 1. Effects of DOCA administration on mean arterial pressure (MAP), thirst, and salt appetite in sheep. DOCA was administered as a subcutaneous implant of Silastic strips impregnated with DOCA (100 mg/kg sheep weight) at time zero. "Day" indicates time after implant. The DOCA treatment was terminated by surgical removal of the implant. "Postimplant" values were obtained 5 days after the DOCA implant was removed. MAP was measured directly from chronically implanted aortic catheters. "Salt appetite" was measured as the ratio of high-salt (266 meq Na/kg) to low-salt (6 meq Na/kg) food selected by the sheep. Data are the means and standard errors obtained from six sheep. Asterisks indicate differences from "preimplant" values in which $P < 0.05$.

ing the DOCA implant period, leveling off after 10 days at a value of 2.88. Following removal of the DOCA implant, the value rapidly fell to 0.54 during the first 5-day period of the recovery. These data indicate that

whereas during the control periods the sheep chose to eat only half as much salty as unsalty alfalfa, during the DOCA treatment period their salt appetite had increased so that they ate about three times as much salty as unsalty food. This change in salt appetite was reversed within 2 days after the termination of DOCA treatment.

In Fig. 1B it is evident that water intake (thirst) followed a course that was similar to the changes in salt appetite. Water intake increased gradually from a control value of 5 liters per day to over 11 liters per day under the influence of DOCA treatment. Following cessation of the treatment, water intake returned to near control values.

Systolic and diastolic pressures rose from control values of $91 \pm 2.2/69 \pm 3.0$ mm Hg to $104 \pm 4.9/83 \pm 2.7$ mm Hg in the first 5-day period, and leveled off at $121 \pm 4.1/98 \pm 3.0$ mm Hg following 2 weeks of DOCA treatment. Five days after removal of the DOCA implants these pressures fell back to control values of $94 \pm 4.1/71 \pm 2.2$ mm Hg. Corresponding direct measurements of mean arterial pressure (MAP) are presented in Fig. 1A. The changes in these values also correspond to the changes in salt appetite and thirst.

Measurements of plasma sodium and potassium concentration during these three periods are presented in Fig. 2. Both of these variables change significantly within 24 hr after either the beginning or cessation of treatment with DOCA. Hypernatremia and hypokalemia persist unabated throughout the treatment period. Hyperkalemia rebounds slightly following the cessation of DOCA treatment.

Discussion. Salt appetite is a psychophysiological trait that has obvious relevance to hypertension. In addition to the recognized relationship between salt intake and hypertension, salt appetite has been observed to increase in response to psychosocial stress (14, 15). It is also greater in spontaneously hypertensive rats (SHR) than in normotensive rats (WKY) (16, 17).

When given a free choice of sodium intake, rats, sheep, and humans appear to have quantitatively similar salt appetites (15). This important regulator of sodium intake is

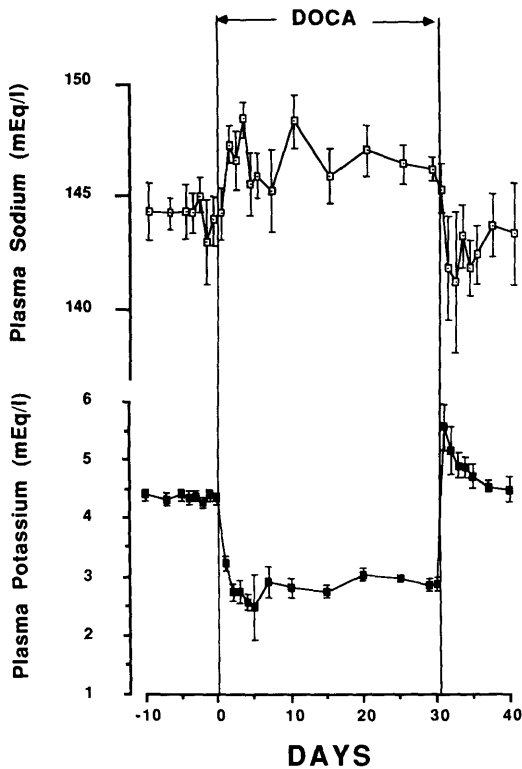


FIG. 2. Effects of DOCA administration on plasma levels of sodium and potassium. Significant hypernatremia and hypokalemia persisted throughout the period that the sheep had the DOCA implant. Both values reverted to normal following removal of the DOCA implant. Data represent the means and standard error of measurements made on six sheep.

usually studied in the rat on a standard diet, with a free choice of water or solutions of different concentrations of NaCl to drink. This type of study is referred to as the multiple bottle "cafeteria preference" technique. Most previous experimental studies of salt appetite have offered a choice of sodium intake in the drinking solutions. To our knowledge, the current study is the first large animal study in which the choice has been given in the food, and its success, based on the uniformity of the results, demonstrates that this is also a satisfactory approach to the study of salt appetite.

Using the multiple bottle technique, Richter (18) demonstrated that rats could regulate their sodium intake to meet their

needs. He observed that adrenalectomized rats would preferentially increase their intake of 1% NaCl solution over water, and would thereby increase their survival. Administration of low doses of DOCA to adrenalectomized rats abolished their augmented salt appetites. Wolf (12) demonstrated that this decrease in sodium intake in the DOCA-treated adrenalectomized rat was due to the return of their ability to retain sodium. When greater doses of DOCA were given to either adrenalectomized or intact rats, these animals also increased their salt appetites (12, 13, 19). This increase did not fulfill any obvious need for sodium.

Denton (20) has developed a sodium-depleted model in the sheep. By transplanting the parotid duct to the skin of the cheek, the animal loses 1 to 4 liters of saliva per day containing 200–700 meq sodium. This procedure elicited a specific sodium appetite. Subsequent studies (21) demonstrated that this response did not depend on the gustatory sense, since it was not abolished by severing the sensory nerves for taste. Furthermore, it was not mediated by changes in the plasma level of sodium since manipulation of this variable did not alter the salt appetite (22).

Extensive studies have explored the dependence of salt appetite on the brain. Andersen *et al.* (23) observed that electrical stimulation of the hypothalamus in sheep elicited an immediate response of licking a salt block. Novakova and Cort (24) studied rats in a sodium-depleted state. Whereas a control group exhibited the expected elevated salt appetite, the group with hypothalamic lesions failed to show this increased salt appetite. Several studies (25–27) have dealt with the complex neurocircuitry involved in the salt appetite. Similar studies have provided extensive insights into the role of the hypothalamus in the DOCA-induced sodium appetite (28).

Fregly (16) has demonstrated that the spontaneously hypertensive rat (SHR) has an elevated sodium-specific salt appetite. This appetite is reversed by a central blockade of the angiotensin II converting enzyme (17). This observation suggests that the central action of angiotensin is involved with salt ap-

petite as it is in thirst and blood pressure regulation (29, 30).

That DOCA acts centrally to cause hypertension is documented by the observation that the standard treatment with this mineralocorticoid fails to cause hypertension in a rat that has been subjected to a specific hypothalamic lesion (31). Furthermore, mineralocorticoids delivered into the lateral cerebral ventricle cause hypertension in a minute dose that does nothing when it is delivered systemically (32).

In the current study we have observed that DOCA administration produces increases in arterial pressure, thirst and salt appetite. During the DOCA-induced increase in salt appetite, the sheep exhibited a drive for sodium during a time when it had no evident need for this ion. In fact, plasma levels of sodium were elevated. Therefore, the elevated salt appetite represents an abnormal and possible deleterious pharmacological effect of DOCA, as does the polydipsia and the hypertension.

At a cellular level mineralocorticoids are known to increase membrane permeability to sodium (33–35). We have observed that DOCA treatment causes an immediate increase in intracellular sodium content in red blood cells (36). We have also found that an increase in lymphocyte membrane permeability to sodium accompanies DOCA hypertension (37). Intralymphocytic sodium content is also increased.

The changes in the three variables reported in the current study appear to be caused by the action of the mineralocorticoid on the hypothalamic regulatory centers for salt appetite, thirst, and blood pressure. On the basis of the known cellular actions of mineralocorticoids, we hypothesize that DOCA changes the activities of these centers by causing an increase in their intracellular sodium content.

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