Inhibition of Aldosterone Secretion by Passive Transfer of Antirenin Antibodies to Dogs on a Low Sodium Diet*† (30166)

T. C. LEE, E. G. BIGLIERI, E. E. VAN BRUNT‡ AND W. F. GANONG
Department of Physiology and Medicine, School of Medicine, University of California, San Francisco Medical Center, San Francisco

The fact that renin and angiotensin II stimulate aldosterone secretion is established (1-3), but the contribution of the renin-angiotensin system to the increase in aldosterone secretion seen in various physiologic states remains uncertain. We have previously found that the plasma of dogs actively immunized with hog renin inhibits the aldosterone-stimulating as well as the pressor activity of both hog and dog renin(4). In an attempt to determine whether increased renin secretion was responsible for the increase in aldosterone secretion produced by restricting sodium intake, the increase produced by a low sodium diet in immunized dogs was measured(5). The response in these animals was normal. However, there was reason to believe that the actively immunized dogs had developed compensatory mechanisms which permitted them to secrete renin in amounts sufficient to affect aldosterone secretion. To avoid the effects of such long-term compensations, we have studied the acute effects on aldosterone secretion of antirenin antibodies passively transferred to dogs on a low sodium diet.

Methods. Mongrel dogs of both sexes were immunized with hog renin, and their plasma antirenin antibody titers followed. The schedule used to produce active immunization of dogs with hog renin, and the method for measuring their plasma antirenin antibody titers have been reported previously(5). When the plasma antirenin antibody titers were found to be elevated, the dogs were anesthetized with pentobarbital and bled 200 to 300 ml each. They were then permitted to recover, and were subsequently treated with renin and bled again. Some were bled 3-4 times before they were sacrificed. The blood was collected in citrate solution. The plasma was separated by centrifugation and frozen for subsequent isolation of the β- and γ-globulin fractions of the plasma proteins by Cohn fractionation(6). These fractionations were carried out by Pentex Co., Kankakee, Ill.

Aldosterone secretion rates were studied in 28 normal, male mongrel dogs weighing 7.3 to 20.2 kg. The dogs were divided into 4 groups of 7 each, as shown in Table I. Two groups (A and B) were placed on a measured diet providing each dog less than 1 mEq of sodium and 20 mEq of potassium per day. The other 2 groups (C and D) received a diet providing each dog 40 mEq of sodium and 20 mEq of potassium per day. The methods for making up these diets have been published previously(5). After 5 days on the prescribed diet, the dogs were weighed, anesthetized with pentobarbital and given γ-globulin from immune dogs (Groups A and C) or control protein fractions (Groups B and D). The fraction for each dog was dissolved in 100-200 ml of 5% dextrose solution and infused intravenously. There was some variation in the concentration of antirenin activity per gram of protein fraction in the different batches. The dogs in Group A received 750-1000 antirenin units in 0.7-2.89 g of protein. Those in Group C received 530 to 1000 antirenin units, in 2.25-11.75 g of protein. Four of the dogs in Group B received 0.16-3.0 g of γ-globulin from normal dogs, and one received 2.3 g of γ-plus 0.8 g of β-globulin from normal dogs, while 2 received 0.6-0.7 g of β-globulin from immunized dogs. Six of the dogs in Group D received 3.0 g of γ-globulin from normal dogs, while one received 2.3 g plus 0.8 g of β-globulin from normal dogs.

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TABLE I. Effect of Passively Transferred Antirenin Antibodies on Aldosterone Secretion in Dogs. Seven dogs in each group. Values are means ± standard error of mean.

Group	Plasma protein injected	Sodium intake, mEq/day	Aldosterone secretion rate, μg/day	Antirenin titer 1 hr after protein infusion, AU/ml
A	Immune γ-globulin	<1	27 ± 2.7	.4 ± .2
B	Control	<1	59 ± 6.5	0
C	Immune γ-globulin	40	31 ± 7.3	.7 ± .1
D	Control	40	32 ± 7.4	0

Blood was drawn before and one hour after infusion of the protein. Approximately 3 μC of aldosterone-1,2 3_H was then administered intravenously for determination of the aldosterone secretion rate. After this injection, the dogs were placed in metabolism cages and 24-hour urine specimens were collected for 3 days. The bladder was emptied by catheterization at the start of the experiment and at the end of each collection. Blood was drawn, and the animals weighed after the last collection. The sodium and potassium content of each urine collection was measured, and the urines from each dog were then pooled. The specific activity of the pH 1 extractable derivative of aldosterone in each pool was determined and the aldosterone secretion rate of each dog was calculated(5,7). The aldosterone secretion rate of the one dog in which the tritium/carbon-14 ratio was less than one was discarded because the error in counting tritium was too great(8). The sodium and potassium contents of the plasma at the beginning and the end of experiment were measured, and antirenin activities in the plasma samples taken before, one hour after, and 3 days after injection of the protein fractions were also measured(6).

Results. The mean aldosterone secretion rates in the 4 groups of dogs are shown in Table I. Values in the dogs given β-globulin from immune dogs or normal dogs were no different from those of dogs given γ-globulin from normal dogs, so they have been grouped with the latter values for calculation of mean values. The aldosterone secretion rate of the dogs on the low sodium diet given immune γ-globulin (Group A) was significantly lower ($P<0.01$) than that of the dogs on a low sodium diet given comparable quantities of control plasma protein fractions (Group B). The secretion rate in Group A did not differ significantly from the aldosterone secretion rates of the 2 groups fed a normal sodium diet (Groups C and D), and the rates in these 2 groups were almost identical. The mean aldosterone secretion rate of the dogs in Group B was significantly different from that of the dogs in Group D ($P<0.02$).

One hour after administration of the antirenin antibodies, the plasma antirenin titers of the dogs in Groups A and C ranged from 0 to 1.5 AU per ml of plasma, with mean values of less than 1 AU/ml in both groups (Table I). The plasma antirenin titers at the end of the third day of urine collection ranged from 0 to 0.3 AU/ml of plasma in these dogs, with most animals showing zero activity. No antirenin activity was detected in any of the plasma samples taken from the dogs receiving the control protein fractions.

Sodium excretion was elevated during the first 24-hour period after injection in the dogs given γ-globulin from immune dogs, but so was sodium excretion in the dogs given control injections (Table II). The mean plasma sodium level at the end of the experiment was significantly lower in Group A than in Group B. The cause of this difference is unknown. However, there were no other clearly significant differences between these 2 groups, nor between Groups C and D. Furthermore, the plasma electrolyte values after injection of the protein did not differ significantly from those before injection in any of the groups.

Discussion. The increased aldosterone secretion rate normally seen in animals fed a low sodium diet was clearly absent in the passively immunized animals of Group A. Instead, the secretion rate in these dogs was in the same range as that of dogs fed 40 mEq of sodium per day. The aldosterone

TABLE II. Sodium and Potassium Excretion, Plasma Electrolyte Levels, and Weight Loss over a 3-Day Period in Dogs. Dogs in Groups A and C received antirenin antibodies and those in Groups B and D received control protein injections. Values are means ± standard error of mean.

Group	Sodium intake, mEq/day	Sodium excretion, mEq/day			Potassium excretion, mEq/day			Plasma electrolytes, mEq/liter				Wt loss on diet, kg
								Sodium		Potassium		
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Before	After	Before	After	
A	<1	10.5 ± 3.5	3.3 ± .7	3.9 ± 1.3	25.7 ± 4.0	13.4 ± 2.9	13.4 ± 2.2	142.7 ±2.6	140.0 ±1.8	3.7 ± .3	4.0 ± .2	.8 ± .2
B	<1	9.7 ± 4.2	1.4 ± .6	3.9 ± 1.7	20.2 ± 1.5	7.7 ± 1.6	16.8 ± 3.1	151.6 ±3.2	147.6 ±1.6	4.4 ± .2	4.5 ± .1	.7 ± .1
C	40	51.1 ± 7.2	27.4 ± 6.2	30.0 ± 4.8	26.4 ± 6.6	14.6 ± 1.1	17.0 ± 1.8	145.9 ±2.5	142.1 ±1.7	4.2 ± .1	4.0 ± .1	.3 ± .1
D	40	39.3 ± 9.6	17.1 ± 4.7	23.4 ± 6.8	28.3 ± 4.8	12.3 ± 2.8	13.9 ± 2.1	142.4 ±1.3	141.6 ±1.8	3.9 ± .3	3.9 ± .2	.7 ± .1

secretion rate in the dogs fed 40 mEq of sodium per day was unaffected by administration of γ-globulin from immune dogs. These observations indicate that the increase in aldosterone secretion produced by dietary salt restriction is mediated *via* the renin-angiotensin system in the dog. The recent demonstration that a low salt diet causes a prompt elevation in circulating renin and angiotensin II levels(9-12) also supports this conclusion. However, the renin-angiotensin system apparently does not contribute to the maintenance of aldosterone secretion when the sodium intake is 40 mEq/day. Dogs fed a commercial dog food in the amount recommended by veterinarians have a sodium intake of approximately 40 mEq of sodium per day (Ganong, unpublished).

The antirenin levels in the plasma of the passively immunized dogs were low (0-1.5 AU/ml) compared to levels of 1-65 AU/ml in actively immunized dogs(5), but the adrenocortical response to salt restriction was inhibited in the former and not in the latter. However, the increase in aldosterone secretion normally produced by constriction of the aorta above the renal arteries is also present in actively immunized dogs, and aortic constriction is known to increase the circulating renin level(13). Therefore, compensatory mechanisms of some sort must be operating to permit the secretion of physiologically significant amounts of renin in the actively immunized animals. Presumably, such compensations do not have time to develop when acute responses of dogs immunized by passive transfer are studied.

The effects of passively transferred antirenin antibodies on aldosterone secretion have not been reported before, but Hartroft (14) has reported that these antibodies cause an increase in sodium excretion in dogs on a low sodium diet. A natriuresis occurred during the first 24 hours after injection in our passively immunized dogs, but a similar response occurred in the dogs infused with protein fractions that had no antirenin activity. Furthermore, the increased sodium excretion was associated with a kaliuresis in all 4 groups of animals. Therefore, the natriuresis could not have been due solely to decreased

aldosterone secretion. Expansion of the intravascular and interstitial fluid volume in the dog is known to raise the glomerular filtration rate and decrease the tubular reabsorption of sodium, causing an increase in sodium excretion that occurs even when the dogs are treated with large doses of mineralocorticoids (15). It seems likely that this mechanism was operating in our dogs, since the osmotic effects of the injected protein undoubtedly caused an expansion of intravascular volume. It should also be noted that when the effects of aldosterone are inhibited by administration of spironolactones to individuals on a low sodium diet, there is only a slight increase in sodium excretion(16). In the present experiments, a small increase in sodium excretion due to decreased aldosterone secretion would have been obscured by the natriuresis that occurred in all 4 groups of dogs.

Summary. Antirenin antibodies were infused intravenously and their effect on aldosterone secretion measured in 7 dogs fed 1 mEq of sodium per day and in 7 dogs fed 40 mEq of sodium per day. The mean aldosterone secretion rate in the dogs on the low sodium diet was significantly lower than that of 7 dogs on the same diet given control injections of protein. However, the mean aldosterone secretion rate in the dogs fed 40 mEq of sodium per day was in the same range as the rate in 7 dogs on the 40 mEq diet given control injections of protein. Furthermore, the value in the group of dogs on the low salt diet that were given the antirenin antibodies was in the same range as the values in these latter 2 groups. These results indicate that the increase in aldosterone secretion produced by restricting sodium intake is mediated *via* the renin-angiotensin system, while basal secretion on a more adequate sodium intake is independent of this system. There was a moderate increase in sodium excretion in the dogs receiving the antibodies, but a similar response also occurred in the dogs given control injections of plasma proteins. This suggests that the increased sodium excretion was not due primarily to inhibition of aldosterone secretion, but to some other natriuretic mechanism.

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