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Plasma Antinatriferic Activity in Hydropenic and Volume-Expanded Dogs: A Blind Study (38773)

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(Introduced by N. S. Skinner, Jr.)

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A number of well-controlled experiments have indicated the existence of a nonadrenal humoral natriuretic substance which is released in response to expansion of the extracellular fluid volume (1–4). Several laboratories, including our own, have demonstrated biologic activities which are compatible with such a humoral natriuretic substance (5–8).

In our own studies, we showed that plasma of dogs with acute expansion of the extracellular fluid volume contains an ultrafilterable inhibitor of anuran membrane sodium transport, which is also natriuretic (7, 8). However, there has been a report that studies utilizing techniques similar to ours have failed to confirm our results (9). Since our original studies were not performed in a blind manner, and because of the question raised concerning the reproducibility of our results, we felt that a totally blind study was advisable. Accordingly, plasma samples collected in two separate, independent laboratories were studied by us for effect on toad bladder short-circuit current in a blind manner. The results obtained in this study were similar to those previously reported (7, 8).

Materials and Methods. Samples of jugular venous plasma were obtained from mongrel dogs of both sexes. Twenty-six samples were collected in laboratory 1 (Mayo Clinic)[1] and eight samples in laboratory 2 (National Institutes of Health)[2]. Plasma was frozen at −4°, coded, and shipped in dry ice to laboratory 3 (VMB) for ultrafiltration and toad bladder assay.

Hydropenic samples were obtained from 12 dogs in laboratory 1. The dogs ingested a diet containing 3 meq per day of sodium for 10 days and were given 15 mg/day of deoxycorticosterone (DOC) intramuscularly. After overnight dehydration, they were anesthetized with pentobarbital sodium, and jugular venous blood was drawn 1–3 hr later.

Fourteen samples from acutely volume-expanded dogs were obtained in laboratory 1. The dogs were deprived of water for 12–14 hr prior to the experiment, anesthetized with pentobarbital, and given 0.9% NaCl equal to 6–10% of body weight in 60 min. Nine of the dogs ingested a diet containing 3 meq Na and five a diet containing 100 meq Na for 10 days prior to the experiment. All were given 15 mg of DOC per day for 10 days. Jugular venous blood was drawn 60 min after beginning saline infusion.

In laboratory 2, a protocol identical to the one reported by laboratory 3 (7) was used. Five dogs were fed a normal diet for several days and were deprived of food and water overnight before the experiment. Anesthesia was induced with intravenous pentobarbital and a hydropenic sample was obtained 60–90 min later. In three of the five dogs, 0.9% saline was given intravenously, 500 ml in 20 min, then 10 ml/min. Jugular venous blood was drawn 60 min after beginning saline.

The blind assay data obtained on samples from laboratories 1 and 2 were compared to nonblind assay data on samples collected from three groups of animals in laboratory 3. Group A consisted of nine dogs in which paired blood samples were obtained during hydropenia and 60 min after beginning volume expansion with 0.9% saline, as previously described (7). The means and standard errors for assays performed on these samples are shown in Fig. 6 in a previous publication (10). Group B consisted of 12 hydropenic dogs not previously published. Group B dogs ingested a normal diet (Purina Lab Chow) for 3 days and were deprived of food and water overnight before the experi-

[1] By Drs. F. G. Knox and E. G. Schneider.

[2] By Dr. R. I. Keimowitz.

TABLE I. PLASMA SAMPLES FROM LABORATORIES 1 AND 2.

Hydropenia[a]			Acute volume expansion[b]		
Sample no.	PD (%)	SCC (%)	Sample no.	PD (%)	SCC (%)
1	−10	−4	1[c]	−14	−33
2	−6	−15	2[c]	−12	−1
3	−3	0	3[c]	−12	−17
4	−1	0	4[c]	−30	−34
5	−6	−14	5[c]	−27	−25
6	−13	−30	6[d]	−19	−24
7	0	+2	7[d]	−9	−24
8	−6	−4	8[d]	−15	−26
9	−14	−31	9[d]	−15	−20
10	−15	−25	10[d]	−2	+1
11	−16	−25	11[d]	−12	−20
12	+1	−18	12[d]	−18	−16
13	+10	−4	13[d]	−16	−24
14	+7	−17	14[d]	−13	−26
15	−11	−19	15	−10	−15
16	−17	−17	16	−23	−25
17	+8	+6	17	−1	−24
Mean	−5	−13		−15	−21
SE	2.1	2.8		1.83	2.3
t				3.015	2.24
P[e]				<.01	<.02

[a] Samples 1–12 from laboratory 1, 13–17 from laboratory 2.

[b] Samples 1–14 from laboratory 1, 15–17 from laboratory 2.

[c] One hundred meq sodium diet plus DOC (see Methods).

[d] Three meq sodium diet plus DOC (see Methods).

[e] Significance of difference in PD and SCC between hydropenia and acute volume expansion.

ment. They were anesthetized with pentobarbital and a jugular venous blood sample was obtained 60–120 min later. Group C consisted of 16 dogs recently reported (8) in which samples were obtained during acute volume expansion with 0.9% saline according to the previously described protocol (8).

Ultrafiltrates of plasma were prepared using PM-10 membranes[3] (MW cut-off 10,000) and tested for their effects on toad bladder short-circuit current by previously described methods (7, 8, 11). Data are expressed as percent change in potential difference (PD) and short-circuit current (SCC) 30 min after addition of ultrafiltrates to the toad bladder chamber. Statitstical analyses were performed using Student's *t* test employing a PDP-8 computer.

[3] Amicon Corp., Lexington, Mass.

Results. The results of toad bladder assays performed in laboratory 3 on blood samples obtained in laboratories 1 and 2 are shown in Table I. Ultrafiltrates of plasma from 17 hydropenic dogs caused a mean change in PD of $-5 \pm 2.1\%$ and in SCC of $-13 \pm 2.8\%$. Ultrafiltrates of plasma from acutely volume-expanded dogs caused a mean change in PD of $-15 \pm 1.8\%$ and SCC of $-21 \pm 2.2\%$. The difference between the hydropenic and volume-expanded group is highly significant. ($P < 0.01$ for PD and <0.02 for SCC).

These results are compared in Table II with data from samples collected in laboratory 3 where nonblind assays were performed. The two hydropenic groups from laboratory 3 caused a mean change in PD of $-5 \pm 5.4\%$ and $-10 \pm 2.5\%$, and in SCC of $-14 \pm 5.6\%$ and $-12 \pm 3.3\%$. These results are not significantly different from each other or from the blind assay results of hydropenic samples shown in Table I. The two saline-expanded groups showed a mean change in PD of $-16 \pm 4.6\%$ and $-17 \pm 2.2\%$, and in SCC of $-28 \pm 4.1\%$ and $-25 \pm 3.1\%$. These two groups are not significantly different from each other or from the blind assay results in the volume-expanded group in Table I.

Discussion. This study demonstrates that assays of dog plasma ultrafiltrates for sodium transport inhibitory activity performed using a blind protocol yield values similar to those previously reported. These results support previous observations showing an ultrafilterable inhibitor of sodium transport in plasma of acutely expanded dogs, and indicate that earlier data were not the result of observer bias.

It should be pointed out that the hydropenic dogs in laboratory 1 were ingesting a low sodium diet and were sodium depleted, in contrast to previous studies in which hydropenic dogs ingested a normal diet. Despite this difference, some chronically sodium-depleted animals appear to have considerable sodium transport inhibitory activity (e.g., sample numbers 6, 9, 10, 11, Table I), as do several of the hydropenic

TABLE II. PLASMA SAMPLES FROM LABORATORY 3.

Group A[a]					Group B[b]			Group C[c]		
Hydropenia			Acute volume expansion		Hydropenia			Acute volume expansion		
Sample no.	PD (%)	SCC (%)	PD (%)	SCC (%)	Sample no.	PD (%)	SCC (%)	Sample no.	PD (%)	SCC (%)
1	+21	+1	−34	−38	1	+3	+8	1	−15	−33
2	−22	−22	−39	−43	2	−17	−9	2	−15	−2
3	−10	−18	−4	−22	3	−14	−4	3	−9	−17
4	+7	+16	−7	−25	4	−21	−26	4	−2	−6
5	−7	0	−12	−27	5	−11	−24	5	−23	−27
6	−31	−30	−15	−25	6	+1	−18	6	−19	−50
7	−8	−17	−5	−11	7	−22	−28	7	−14	−17
8	−6	−23	−2	−12	8	−2	−4	8	−22	−30
9	+11	−36	−28	−45	9	−13	−16	9	−20	−26
					10	+1	−2	10	−5	−13
					11	−12	−9	11	−15	−33
					12	−9	−9	12	−25	−29
								13	−32	−42
								14	−10	−26
								15	−30	−30
								16	−18	−21
Mean	−5	−14	−16	−28		−10	−12		−17	−25
SE	5.37	5.60	4.6	4.1		2.5	3.1		2.1	3.1
P[d]	>0.4	>0.3	>0.3	>.06		>0.09	>0.4		>0.9	>0.1

[a] Data are obtained from hydropenic and saline-loaded dogs previously reported (10) in which only means and standard errors were published.

[b] Data are from hydropenic dogs studied in laboratory 3 not previously reported.

[c] Data are from volume-expanded dogs recently reported (8).

[d] Significance of difference between hydropenic and volume-expanded groups in Table II versus similar groups shown in Table I.

animals on a normal diet (groups A and B, Table II).

The significance of this activity in hydropenic and sodium depleted animals is not clear. We have previously suggested that the sodium transport inhibitory activity in hydropenic dogs on a normal sodium intake represents that activity which may be contributing to the day-to-day adjustment of sodium excretion to sodium intake (10). The finding of the present study in animals on a very low sodium intake excreting virtually no sodium in the urine raises doubts about that interpretation. However, it should be noted that we have found no detectable transport inhibitory activity in acutely volume-depleted dogs after furosemide administration (8). Thus, there is a highly significant difference between the SCC inhibition in Group B, Table II, of $-12 \pm 3.1\%$ and that found in a group of acutely volume-depleted dogs of $2 \pm 1.9\%$ previously reported ($P < 0.01$) (8). This suggests that the absence of sodium transport inhibitory activity correlates more closely with acute rather than chronic decreases in extracellular fluid volume.

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