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Augmented Natriuretic Response to Infusion of Saline in Dogs Rendered Acutely Hypertensive with Metaraminol. (31256)

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Hypertensive subjects respond to infusion of sodium with an exaggerated natriuresis(1). This response is observed in patients with essential hypertension(2,3) and also in those with hypertension due to Cushing's syndrome (4,5), primary aldosteronism(6,7), pheochromocytoma(5) or unilateral renovascular disease(8). This natriuresis therefore appears to be a nonspecific response related only to the presence of elevated blood pressure. Indeed normal subjects exhibit an augmented natriuretic response to infusion of sodium when a temporary hypertensive state has been pharmacologically induced by administration of metaraminol(9).

The mechanism by which human hypertension alters the renal response to sodium loading is not known and no intact animal model has been available for study of this phenomenon(10). In the present study an attempt has been made to determine if animals rendered hypertensive by metaraminol respond to saline loading with a greater increase in urinary sodium output than occurs when the same animals are normotensive. The data demonstrate that dogs exhibit an enhanced natriuretic response to infusion of sodium during pharmacologically-induced hypertension.

Methods. Six non-pregnant mongrel bitches were maintained on a constant diet without restriction of fluid. Studies were performed in the morning after food and fluids had been withheld for 18 hours. Dogs were anesthetized with pentobarbital, 30 mg per kilogram, which was supplemented as necessary during the study. Aqueous vasopressin, 5 u, was

given subcutaneously ½ hour prior to study and was continued intravenously with the sustaining infusion at a rate of 4 milliunits per minute. Blood pressure was determined with a mercury manometer connected to a femoral artery catheter and arterial samples were withdrawn at appropriate intervals for analysis. Urine samples were obtained through an indwelling bladder catheter using air washout.

A urine collection was obtained in the absence of any infusion for determination of control values for creatinine clearance and sodium excretion. Inulin and paraaminohippurate (PAH) were not given in order to avoid observing effects of infusion during the control period. Following this control collection priming injection of inulin and PAH was given to make possible subsequent measurement of glomerular filtration rate and renal plasma flow. An infusion of normal saline containing appropriate amounts of inulin and PAH was then delivered at 1.0 ml per minute to observe the response to administration of sodium. After 20 minutes had been allowed for equilibration, another urine collection was obtained for a 60-minute period during continued infusion of saline.

Each animal was restudied after an interval of about one week. During this study metaraminol bitartrate was infused during the saline infusion at an approximate rate of 53 μg per minute (.0053 ml per minute) in order to maintain mean BP at about 180 mm Hg. In 3 dogs an additional study was performed during which metaraminol alone was infused but no saline was given. The sequence of the 3 types of study was varied in different dogs.

To determine whether exaggerated natriuresis may result from decreased secretion of a sodium retaining steroid, additional studies were obtained in 4 other dogs. One-half hour prior to these studies an intramuscular injection of 10 mg of desoxycorticosterone acetate (DOCA) was given. In addition, there was added to the priming injection of inulin and PAH 0.5 mg of 2α-methyl-9α-fluorocortisol* and this compound was continued with the sustaining infusion at a rate of 2 μg per minute. The study protocol was otherwise unaltered in these animals.

Analyses for osmolality, sodium, potassium, inulin and PAH were performed as previously described(11) and endogenous creatinine was determined by the method of Brod and Sirota as modified by Smith(12). Excretion rates and clearance values were corrected for a surface area of one square meter.

Results. Data obtained during infusion of saline alone, metaraminol alone, and during infusion of saline with metaraminol in 6 dogs are presented in Table I. Basal excretion of sodium ($U_{Na}V$) was similar during the preinfusion control periods of normotensive and hypertensive studies ($U_{Na}V$ 52 μEq/minute and 63 μEq/minute, respectively). Infusion of sodium resulted in decreased sodium excretion in 2, and minimal increases in 4 of the 6 dogs when normotensive (mean change plus 15 μEq/minute). Marked natriuresis occurred in all 6 dogs when hypertensive (mean increment plus 207 μEq/minute). The increased renal output of sodium during saline infusion was significantly greater ($P<.01$) when the dogs were hypertensive than when blood pressure was normal (Table I). Corresponding augmentation of urine flow (V) and osmolar clearance (C_{osm}) was also noted. In fact urine flow was noted to increase abruptly during sodium infusion as the blood pressure became elevated.

Three dogs which showed an augmented natriuretic response to sodium infusion during administration of metaraminol were restudied during infusion of metaraminol alone. A modest natriuresis (of 34 and 45 μEq/minute, respectively) resulted in 2 dogs while sodium excretion decreased in the third.

Serum sodium concentration (P_{Na}) tended to decline slightly during saline infusion.

Potassium. The excretion of potassium (U_KV) tended to increase during sodium infusion. Of 5 dogs in which data are available 3 had greater kaliuresis in the hypertensive state in response to sodium loading. The dog whose $U_{Na}V$ was not augmented during the hypertensive state also failed to show enhancement of U_KV. In the 2 dogs in which metaraminol caused modest natriuresis when infused alone, kaliuresis also occurred.

* Kindly supplied by Upjohn Co., Kalamazoo, Mich.

TABLE I. Effects in Dogs of Administration of Saline Alone, Metaraminol Alone, and Saline with Metaraminol.

Dog #	Type of study	Infusion	V, ml/min	C_{cr}, ml/min	C_{in}, ml/min	C_{PAH}, ml/min	C_{osm}, ml/min	$U_{Na}V$, μEq/min	$U_{K}V$, μEq/min	P_{Na}, mEq/l	Mean BP, mm Hg
1	Normotensive; saline	None	.22	59			1.2	70	11	148	140
		Saline	.40	59	81	177	1.8	123	40	147	140
1	Hypertensive; "	None	.23	75			1.0	84	11	149	140
		Saline and metaraminol	.43	68	111	220	1.4	141	31	149	180
2	Normotensive; "	None	.62	55			1.1	35	37	146	140
		Saline	.46	46	50	150	1.0	30	43	147	140
2	Hypertensive; "	None	.46	56			1.4	96	9	148	150
		Saline and metaraminol	1.2	55	97	193	2.5	246	32	147	185
3	Normotensive; "	None	.28	77			1.4	38		145	130
		Saline	.22	54	101	228	1.2	28	39	138	130
3	Hypertensive; "	None	.08	75			.51	10	11	155	140
		Saline and metaraminol	.96	69	86	188	2.6	223	94	152	180
3	Hypertensive; control	None	.16	57			.56	30	13	155	140
		Metaraminol	.30	74			1.5	64	32	154	180
4	Normotensive; saline	None	.17	60			.90	52	18	134	140
		Saline	.22	64	76	188	1.3	76	25	133	140
4	Hypertensive; "	None	.17	49			.68	30	16	150	140
		Saline and metaraminol	1.5	55	74	178	2.8	275	69	148	180
4	Hypertensive; control	None	.08	37			.33	8	9	148	135
		Metaraminol	.10	39			.42	1	4	148	180
5	Normotensive; saline	None	.25	109			1.5	112	21	153	130
		Saline	.58	87	111	221	2.3	226	29	151	125
5	Hypertensive; "	None	.34	103			2.0	144	9	151	135
		Saline and metaraminol	1.9	73	97	213	4.3	524	32	146	180
6	Normotensive; "	None	.08	56			.52	4	12	149	140
		Saline	.20	77	110	222	1.4	16	71	148	135
6	Hypertensive; "	None	.12	57			.82	14	6	154	140
		Saline and metaraminol	.80	58	77	145	2.4	210	62	154	180
6	Hypertensive; control	None	.09	76			.52	12	10	144	125
		Metaraminol	.32	84			1.6	57	62	153	180

Glomerular filtration rate. Of the 5 dogs which exhibited an exaggerated natriuretic response the glomerular filtration rate (GFR) as estimated from the clearance of endogenous creatinine (C_{cr}) decreased in 3 and increased in 2 during saline infusion when the animals were normotensive (Table I). During infusion with saline and metaraminol, C_{cr} increased in 1, was unchanged in 2 and decreased in 2. C_{in}, which was only measured during saline infusion, was greater during normotensive study in 3 dogs (No. 3, 5, 6, Table I), greater during hypertensive study in 2 dogs (No. 1 and 2) and similar on both occasions in 1 (No. 4).

Renal plasma flow. Renal plasma flow (RPF) was estimated from the PAH clearance (C_{PAH}) during infusion of saline. C_{PAH} averaged 198 ml/minute when saline alone was given and 190 ml/minute during infusion of metaraminol with saline.

Study during administration of antinatriuretic hormones. Data obtained in 4 dogs studied while receiving DOCA and 2α-methyl-9α-fluorocortisol are presented in Table II. Three animals failed to exhibit greater augmentation of $U_{Na}V$ in the hypertensive state. The remaining dog (No. 9) demonstrated enhanced natriuresis in response to saline infusion when hypertensive, despite the presence of sodium-retaining steroids.

Discussion. In the present study it has been shown that dogs made acutely hypertensive with metaraminol exhibit enhanced natriuresis in response to saline infusion. An augmented natriuretic response to sodium loading in hypertensive states is thus demonstrated to occur in a species other than man.

Glomerular filtration rate (as measured by endogenous creatinine clearance) was not augmented to any greater extent when metaraminol was administered during saline infusion than when saline was given alone. In fact the filtered load of sodium tended to decrease during the augmented natriuretic response. Inulin clearance (determined during saline infusion) further confirms that GFR was not consistently higher on the day the dog was hypertensive than on the day she received saline alone. It thus appears that changes in filtered load of sodium cannot account for the

TABLE II. Effects in Dogs Receiving DOCA and 2α-Methyl-9α-Fluorocortisol of Administration of Saline Alone and Saline with Metaraminol.

Dog #	Type of study	Infusion	V, ml/min	C_{cr}, ml/min	C_{in}, ml/min	C_{PAH}, ml/min	C_{osm}, ml/min	$U_{Na}V$, μEq/min	U_KV, μEq/min	P_{Na}, mEq/l	Mean BP, mm Hg
7	Normotensive; saline	None	.51	70			.91	55	20	149	135
		Saline	.64	63	74	191	1.3	95	38	149	130
7	Hypertensive; "	None	.077	74			.48	5	2	148	135
		Saline and metaraminol	.26	74	66	177	1.4	57	36	147	180
8	Normotensive; "	None	.13	59			.74	13	7	148	145
		Saline	.20	75	72	244	1.4	51	18	146	140
8	Hypertensive; "	None	.11	65			.75	9	14	146	140
		Saline and metaraminol	.23	55	90	160	.97	15	35	146	180
9	Normotensive; "	None	.47	87			1.6	162	9	142	125
		Saline	.30	78	107	350	1.6	108	31	142	130
9	Hypertensive; "	None	.47	66			1.8	145	34	149	125
		Saline and metaraminol	1.6	66	102	272	3.3	346	57	146	180
10	Normotensive; "	None	.21	58			.47	4	18	143	105
		Saline	.30	57	76	113	.80	7	45	143	100
10	Hypertensive; "	None	.21	75			1.0	4	31	137	105
		Saline and metaraminol	.31	65	102	165	1.1	10	55	134	160

enhanced excretion of sodium observed in the hypertensive state. These data are therefore consistent with observations in man which indicate that exaggerated natriuresis may occur despite decreased filtered load of sodium(13). A tubular mechanism must therefore be postulated.

Increases in P_{Na} are known to inhibit tubular reabsorption of sodium and can therefore result in natriuresis(14,15). This mechanism cannot be invoked in the present study which indicates that in animals, as in man(16), exaggerated natriuresis may occur despite decreasing blood levels of sodium.

Only 1 dog in 4 exhibited exaggerated natriuresis in the presence of exogenous sodium-retaining steroids. It thus remains possible that the observed augmented natriuretic response to sodium loading in hypertensive dogs is due to inhibition of adrenal hormone secretion. However, natriuresis in normal dogs after rapid sodium loading is known not to depend on such hormonal inhibition(17,18). Since the antinatriuretic effects of intravenous aldosterone persist for several hours after administration has been stopped(19), suppression of secretion of this hormone could not account for the prompt natriuretic response observed in the present study. The finding that kaliuresis often accompanies the natriuresis further argues against inhibition of adrenal steroids. The failure in the present study consistently to observe an exaggerated natriuretic response in hypertensive dogs treated with antinatriuretic steroids may merely indicate, then, that the factors causing exaggerated natriuresis can be overridden.

Since total body sodium may be augmented in hypertensive subjects(20) the possibility arises that the exaggerated natriuretic response may result from the shedding of excess sodium stores in response to sodium loading. In the present study, however, increased urine flow reflecting the onset of natriuresis was observed as soon as the acute rises in blood pressure were induced, and the amount of sodium given during the equilibration period was exceeded by the amount excreted in the subsequent augmented natriuresis. It therefore appears that in the present instance enhanced natriuresis could not have resulted from excretion of abnormal stores of sodium which were sequestered in the hypertensive state.

Summary. The mechanism of the exaggerated natriuretic response to saline infusion exhibited by hypertensive subjects is not understood. An attempt was therefore made to reproduce this phenomenon in laboratory animals rendered hypertensive by pharmacologic means. Six mongrel dogs were studied. Their natriuretic response to saline infusion at a rate of 1 ml per minute was determined. The same dogs were restudied while their blood pressure was elevated with metaraminol. An enhanced natriuretic response to normal saline infusion was observed in 5 out of the 6 dogs made acutely hypertensive with metaraminol. The demonstration that exaggerated natriuresis may be induced in a species other than man makes available an experimental model for further study of this phenomenon. The present observations support previous evidence in man that exaggerated natriuresis depends on the presence of elevated blood pressure alone and is independent of the cause of the hypertension. From the acute nature of the present experiment the thesis that exaggerated natriuresis represents the shedding of sodium sequestered during the hypertensive state is rendered untenable.

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A High Titered Hemagglutinin in Tissue Culture Prepared from Japanese B Encephalitis Virus.* (31257)

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Since tissue culture (TC) methods were introduced in arbovirus work, may have attempted to utilize infected fluid as a source of hemagglutinin (HA) antigen as a substitute for tissues and the tedious processing these require in the *in vivo* method currently employed(1). Non-specific inhibitors in certain sera used in TC work were found to mask HA activity, and techniques such as acetone extraction, protamine sulphate or genetron treatment were described for recovering active hemagglutinin(2-4). Other workers used inhibitor-free media or subsequent concentration to achieve higher hemagglutinin titers(5,6); still the HA titers seldom exceeded 1:64.

In search for the optimum method to obtain a Japanese B encephalitis (JBE) virus of high titer from hamster kidney cell (HKC) culture for vaccine purposes, hemagglutinins were explored(7). In many trials with a variety of media including those with serum albumin, it had been found that a minimum of 2% whole serum (usually calf) appeared to be essential to maintain the cells adequately during the early growth phase of the virus to obtain maximal virus titers(8). However, it was found possible when cytopathic effect (CPE) was first noted to remove this serum containing medium, wash the cells, replace with many varieties of serum-free media, harvest 12 to 16 hours later and still recover equally high infectivity titers(7,8). This also provided a direct, easy method for producing high titered, stable hemagglutinin from infected HKC. The relationship of hemagglutinin to infectivity titers was studied.

Materials and methods. Virus strain used: An attenuated strain of JBE virus, designated as OCT-541, line 35-24, plaque 4-5, was used. This is a strain adapted to grow at relatively low temperatures in HKC through many serial passages(9,10).

Preparation of virus suspension: The virus was grown in HKC monolayers in 3 oz bottles at 30°C in the presence of maintenance medium previously described(7), but containing in part 4% normal calf serum (NCS) and lactalbumin hydrolysate. When CPE started to appear the fluid was removed, cell sheets were washed thoroughly with Hanks' balanced salt solution (BSS) and bottles replenished with 7.0 ml of one of a variety of media.

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