Effect of Vasopressin on Calcium and Sodium Excretion in Hydrated Normal Subjects.* (30281)

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Thorn(1), Dicker and Eggleton(2), and Nielsen(3) have suggested from their experiments that the antidiuresis caused by exogenous Lysine-8-Vasopressin and Arginine-8-Vasopressin and by endogenous Vasopressin is associated with a *calcium diuresis*. The latter could be related in a basic way to the mechanism by which antidiuretic hormone (ADH) increases the permeability of the distal portions of the nephrons to water. In the isolated toad bladder Petersen and Edelman (4) demonstrated that a high calcium concentration can inhibit the action of Vasopressin on water permeability without affecting the change in sodium transport; and this inhibition could be overcome by increasing the concentration of Vasopressin in the medium. Using an *in vitro* toad bladder preparation, Bentley(5) found an increased permeability to water when the calcium in the bathing Ringer's solution was below 0.027 mM in the absence of Pituitrin®. The water permeability in the presence of Pituitrin was decreased when the calcium was reduced below 0.27 mM.

However, contrary to the work of Thorn, Heaton and Hodgkinson(6) found no correlation between urine flow and calcium excretion in hydrated normal subjects. Because of these conflicting results and the importance of this possible calcium diuresis to the hormone's action, the present experiments were undertaken.

Materials and methods. Nine healthy men from 25 to 35 years of age were studied. At 8:00 A.M., after an overnight fast, they drank 20 ml/kg body weight of water over 10 minutes. A positive water balance of 20 ml/kg was maintained by the continuous intravenous infusion of 2.5% dextrose solution. With the exception of the 2 experiments described below, the subjects lay quietly in bed throughout the study, standing only to void at 10- to 15-minute intervals. When a maximal sustained water diuresis was attained (60 to 100 minutes after the water-load), the hormones were injected through the I.V. tubing. In 5 subjects alternating doses of Arginine-8-Vasopressin (3.6 to 10.7 mU) and Lysine-8-Vasopressin (3.6 to 11.6 mU) were given by acute injection each followed by an 8- to 23-minute continuous infusion in order for the responses to fall between minimal and maximal antidiuresis. These subjects received an average of 4 injections over a 6-hour period. Two subjects were given a constant infusion of Lysine-8-Vasopressin at 3 different hormone levels (200, 300 and 400 μU/min) for a 60-minute period (Fig. 1). Two subjects were given 20 mU of Pitressin in 10 minutes, exactly according to the method of Dicker and Eggleton(2), with the subjects sitting during the study. The urine was collected in acid-washed test tubes with one drop of concentrated HCl added. Creatinine in the urine was measured by the Jaffe reaction(7). Urinary sodium and calcium were analyzed by the Zeiss spectrophotometer PMQ II with a flame attachment and with a double monochromator MM 12, using acetylene and O_2 for calcium and H_2 and O_2 for sodium. The diluting solution for the urinary calcium contained phosphate, NaCl, and $HClO_4$, according to the method of MacIntyre(8). Urinary osmolality was measured by freezing point depression, using the Fiske Osmometer.

Instead of expressing the excretion of calcium and sodium as a rate in μg and μEq/

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min respectively, they were given as a ratio, calcium and sodium/creatinine, to correct for incomplete bladder emptying observed at low levels of urine flow in some instances.

The antidiuretic response was expressed as per cent reduction in free water clearance (C_{H_2O}) from the baseline diuresis. Because of gradual change in maximal flow, due to diurnal variation, it seemed appropriate to correct this by drawing a hypothetical line between the mean maximal diuresis before and after the hormone was administered. This hypothetical line represents the change in maximal urinary flow which would have occurred if no ADH was administered.

$$\% \text{ reduction} = \frac{\text{hypothetical } C_{H_2O} - C_{H_2O} \text{ during antidiuresis}}{\text{hypothetical } C_{H_2O}} \times 100$$

Results. There was no increase in excretion of calcium following the acute injections or the continuous infusions of Arginine-8-Vasopressin or Lysine-8-Vasopressin, though the free water clearance decreased from 4 to 112%. In Table I during 30 hormone administrations, only 7 showed any increase in calcium excretion. The likelihood of so few increases if there were a true increase by the sign test is $p<.01$. In fact, there may have been a slight downward trend of calcium excretion which accompanied the sodium excretion and may present a diurnal pattern. Fig. 1 shows the lack of overall changes in calcium and sodium excretion related to antidiuresis in 3 constant infusions in 2 subjects with Lysine-8-Vasopressin. Fig. 2 shows the lack of correlation between calcium excretion and antidiuresis in a composite spot graph from all the cases.

The inhibition of endogenous Vasopressin, following the oral and sustained water-load in 4 of the subjects studied, did not affect calcium excretion in spite of going from very concentrated to very dilute urines.

In one of the subjects (M.J.) following the first injection there was a marked rise in both calcium and sodium excretion, which persisted after the effect on C_{H_2O} had disappeared. However, 3 subsequent injections of comparable doses caused no increase in calcium excretion in the same subject.

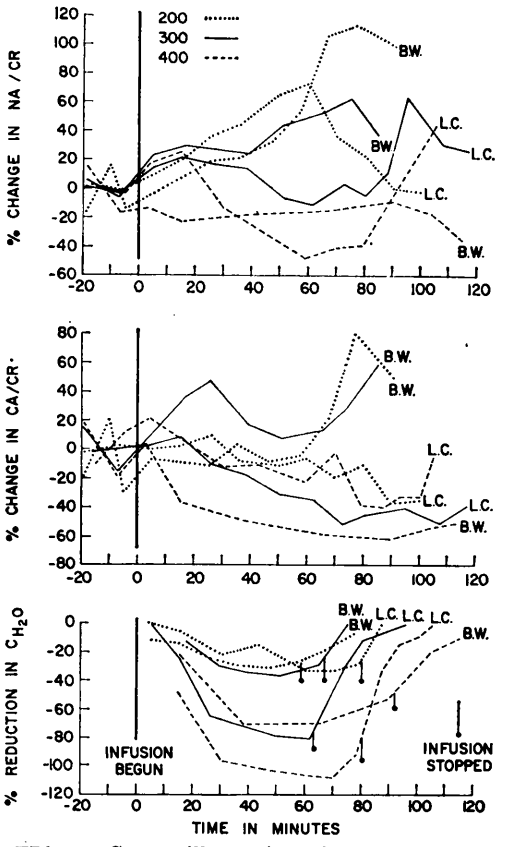

FIG. 1. Curves illustrative of per cent change in Ca/Cr and Na/Cr and per cent reduction in free water clearance during continuous infusion of 200, 300, 400 μU/min of Lysine-8-Vasopressin in 2 subjects. No significant variation in calcium and sodium was observed during periods of antidiuresis.

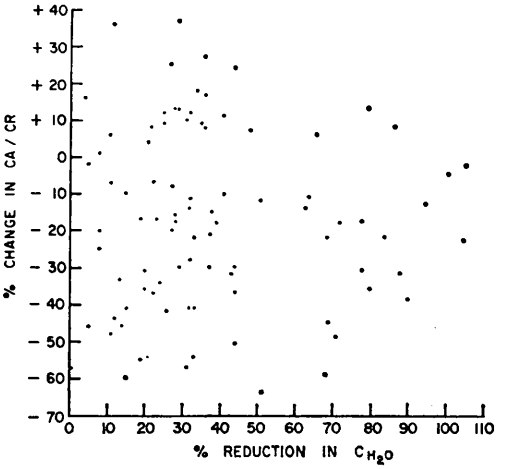

FIG. 2. Correlation between variation in calcium excretion (Ca/Cr) and reduction in free water clearance (C_{H_2O}) by exogenous Vasopressin.

Discussion. Thorn(1) found an increased calcium and magnesium excretion during administration of Vasopressin in hydrated rats and dogs. Unfortunately sodium excretion was not studied. Hydrated rats and dogs have a saluresis under the influence of Vasopressin(9). The changes in calcium excretion might well have been secondary to the increased excretion of sodium. This relationship has been conclusively demonstrated(10, 11). However, in the human no consistent effect of electrolyte excretion has been found following administration of Vasopressin(12), and therefore it is difficult to compare electrolyte excretion in animals and man.

Dicker and Eggleton(2) found increased calcium in the urine when normal human subjects received approximately 20 mU of Vasopressin. In 2 of our subjects we reproduced their experimental design and found no increase. These results were comparable to those of our other experiments. A possible explanation for the discrepancy may have been the different methods employed to determine calcium. Thorn, Dicker and Eggleton, and Nielsen used the complexometric

TABLE I. Percent Change in Excretion of Ca/Cr and Na/Cr After Various Doses of Vasopressin.

Subject & wt, kg	[1]	Dose[2] in mU Sudden	Infusion	C_{H_2O}, % reduction	Ca,[3] μg/min	Ca/Cr,[4] % change	Na,[3] μEq/min	Na/Cr,[4] % change
C.S.	A*	4.85	.97 (8)	32	65	−14	45	−33
86	A	4.85	.97 (8)	38	100	−23	70	−21
	A	9.70	1.94 (8)	65	90	−10	50	−45
	L	5.82	1.16 (8)	28	110	− 1	60	− 6
	L	11.64	2.32 (8)	35	110	+ 5	70	−14
J.A.	A	4.44	.89 (2)	29	45	− 6	205	+ 4
79	A	4.44	.89 (8)	36	40	− 2	185	− 7
	A	8.88	1.78 (8)	48	25	− 6	140	− 1
	L	5.32	1.06 (8)	31	55	−22	135	−25
	L	10.64	2.12 (8)	32	20	+13	125	− 1
R.E.	A	5.34	1.07 (8)	20	95	+12	165	− 2
95	A	10.68	2.14 (8)	49	30	+14	115	− 1
	L	5.34	1.07 (8)	16	60	− 5	150	− 8
	L	10.68	2.14 (8)	30	45	−12	125	−15
M.J.	A	3.57	1.98 (18)	39	95	+69	110	+72
64	A	5.7	3.17 (18)	86	10	−25	85	−13
	L	3.57	1.98 (18)	4	60	− 1	95	− 5
	L	5.7	3.17 (18)	38	40	−28	85	− 2
	L	9.14	5.08 (18)	65	15	− 1	115	−20
V.W.	A	3.71	2.53 (23)	57	50	+26	30	−12
67	L	3.71	2.53 (10)	54	25	−17	35	+ 4
	L	3.71	2.53 (23)	65	25	−15	30	+35
L.C.	L		200 μU/min	32	30	+12	95	+41
	L		300 "	79	20	−57	85	−20
	L		400 "	107	10	− 3	100	−49
B.W.	L		200 "	30	30	−28	85	+ 3
66	L		300 "	35	15	− 9	105	+ 7
	L		400 "	70	25	−36	110	+ 3
L.M. 70	P		20 mU (10)	112	60	−10	285	−13
L.F. 80	P		20 mU (10)	62	185	+12	250	+ 1

r Ca/Cr:C_{H_2O} = −0.29. r Na/Cr:C_{H_2O} = −0.18.

Legend

[1] A = Arginine-8-Vasopressin; L = Lysine-8-Vasopressin; P = Pitressin.

[2] No. in parentheses is length of time infusion was given.

[3] Average rate of electrolyte excretion over each period studied.

[4] % variation of ratios in relation to the baseline during maximal period of antidiuresis.

* Endogenous release of Vasopressin at time of hormone administration.

titration with EDTA, whereas in the present experiment flame photometry by the method of MacIntyre was used(8). In addition Nielsen injected large "unphysiologic" doses of Vasopressin (0.6 to 1.4 units) in his experiments demonstrating an increased calcium excretion(3).

Heaton and Hodgkinson(6) found no relationship between urinary flow and calcium excretion during water load in normal subjects. It is of interest that they measured calcium by flame photometry, a method comparable to the present study. In patients who had therapeutic hypophysectomy for metastatic carcinoma, Gardner *et al*(13) found that water diuresis and Vasopressin-induced antidiuresis did not correlate with calcium excretion. These authors(13) also used a flame photometric methd for analysis. However, the malignant disease and hypophysectomy makes their experiments considerably more difficult to interpret.

The slight decrease in calcium and sodium excretion observed in the present experiments may represent diurnal variation or very slight changes in glomerular filtration rate (Table I, Fig. 2).

Summary. In 9 healthy men doses of Arginine-8-Vasopressin and Lysine-8-Vasopressin from 5.5 to 20 mM which produced decreases in free water clearance (C_{H_2O}) of 4 to 112% had no significant effect on calcium or sodium excretion.

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TRIC Agents Isolated in the United States. X. Immunofluorescence in the Diagnosis of TRIC Agent Infection in Man.* (30282)

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Among the millions of persons afflicted with trachoma the best hope for effective treatment is early diagnosis. For the experienced ophthalmologist the typical case of trachoma (TR) or of inclusion conjunctivitis (IC) is relatively easy to diagnose. There remain many cases in which clinical diagnostic signs are inadequate, and in which laboratory diagnostic tests are needed. To date, the laboratory has been unable to offer adequate help. The most commonly used method has been the demonstration of the typical Giemsa-staining inclusions within epithelial cells of the conjunctiva, first described by Halberstaedter and von Prowazek(1). After Rice(2) demonstrated an iodine-stain-

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