Demonstration of a Defect in the Formation of Adenosine 3',5'-Monophosphate in Vasopressin-resistant Diabetes Insipidus

NORMAN H. BELL,^[25] CHARLES M. CLARK, JR., SUSAN AVERY, TUSHAR SINHA,
CARL W. TRYGSTAD, AND DONALD O. ALLEN

Departments of Medicine, Biochemistry, Pediatrics, and Pharmacology, Indiana University School of Medicine, and Veterans Administration Hospital, Indianapolis, Indiana, USA

Extract

Previous studies had indicated that the effects of vasopressin to increase the permeability of collecting ducts to water and to produce a concentrated urine are mediated through adenosine 3',5'-monophosphate (cyclic AMP). An investigation was performed to determine whether the lack of response to vasopressin in nephrogenic diabetes insipidus could result from an abnormality in the formation of cyclic AMP. The influence of vasopressin was compared in four water-loaded normal subjects, one patient with vasopressin deficiency, and three patients with nephrogenic diabetes insipidus. Serum and urine osmolality, inulin clearance, and cyclic AMP in urine were determined while 5% dextrose and water was infused at a constant rate before, during, and after administration of vasopressin for 1 hr. Vasopressin given intravenously for 1 hr significantly increased the mean rate of excretion of cyclic AMP in urine from a range of from 1.7 to 3.1 nmol/min before treatment to a range of from 4.7 to 8.9 nmol/min after treatment in the normal subjects and the patient with vasopressin deficiency. There were associated mean increases in urine osmolality from a range of from 39 to 119 mOsm/kg to a range of from 568 to 996 mOsm/kg. In contrast, mean cyclic AMP in urine varied from 0.7 to 1.5 nmol/min and mean urine osmolality varied from 47 to 68 mOsm/kg in the patients with nephrogenic diabetes insipidus and did not change after vasopressin. The findings are interpreted to mean that there is a defect in the adenylate cyclase system in nephrogenic diabetes insipidus. It is not known whether there is an abnormality of the receptor, the enzyme, or both or if there is an associated abnormality of the renal tubules to respond to cyclic AMP.

Speculation

The results show that patients with nephrogenic diabetes insipidus do not increase levels of cyclic AMP in urine or concentrate their urine in response to vasopressin. It is proposed that there is a defect in the adenylate cyclase system in these patients which accounts for their lack of response to antidiuretic hormone.

Introduction

Vasopressin-resistant or nephrogenic diabetes insipidus is a familial disease characterized by an inability of the

kidneys to respond to endogenous or exogenously administered antidiuretic hormone [10, 19, 20]. Classically it occurs in males and is inherited as a sex-linked, recessive characteristic [10, 20].

There is evidence that the renal effects of antidiuretic hormone are mediated through the adenylate cyclase system [4, 5, 12, 18]. The question arises, therefore, of whether patients with vasopressin-resistant diabetes insipidus are able to produce cyclic AMP in response to vasopressin. In the present report, the effects of vasopressin on cyclic AMP in urine and renal concentration have been studied. Evidence is presented that patients with vasopressin-resistant diabetes insipidus apparently have a defect in the formation of cyclic AMP in response to vasopressin [2, 9].

Methods

The six patients, all of whom were males, ranged in age from 4 to 52 years. The normal subjects were adult male volunteers who ranged in age from 22 to 24 years. The patients and normal subjects were given a regular diet and water *ad libitum* before each clearance study.

Clearance studies were carried out after an overnight fast. Subjects were kept recumbent and were allowed to sit or stand to void. Except in *patient DR*, in whom the infusion rate was either 2 or 4 ml/min, 5% dextrose in water was infused at a constant rate of 5 ml/min with a Holter infusion pump. In all studies, the infusate was given for at least 30 min before the first control period to allow equilibration. The duration of the clearance periods was 15 min. In the adults, blood samples were obtained at the midpoint of each period. In the children, one sample was obtained at the midpoint of at least one clearance period in each of the control, treatment, and postcontrol treatment periods.

Cyclic AMP was obtained from Schwarz/Mann [21]. It was sterilized by passage through a Millipore filter as described by Levine [15]. It was then administered by constant infusion in 5% dextrose in water as described above.

Serum and inulin in urine were measured by the method of Davidson *et al.* [7, 8]. Serum and urine osmolality were determined by freezing point depression with a Fiske osmometer. Cyclic AMP in urine was determined by the method of Gilman [11] as previously described [1]. Free water clearance (C_{H_2O}) and osmolal clearance (C_{osm}), were calculated with conventional formulas and are expressed as milliliters per minute.

Results

Six patients were studied (Table I). Two of them were brothers (*DR* and *TR*) with nephrogenic diabetes insipidus who had two maternal uncles with the disease. In both generations only the male siblings were affected. A third patient, *RM*, had apparently developed vasopressin unresponsiveness after a febrile illness during his first year of life. These patients did not concentrate their urine after vasopressin. Three patients had vasopressin-responsive diabetes insipidus. One of them, *LB*, had developed the disease after treatment for acromegaly and another one, *RS*, after treatment for chromophobe adenoma. The third patient, *MD*, developed his disease after a severe head injury. After fluid restriction for 18 hr, *LB* had lost some 3.3% of his body weight and had a serum osmolality of 293 mOsm/kg and an abnormally low plasma arginine vasopressin value of 1.3 pg/ml as determined by radioimmunoassay [17], although he was able to concentrate his urine reasonably well. His lack of ability to secrete vasopressin was confirmed in other studies in which hypertonic saline was administered. The patient had polyuria and polydipsia which required treatment with Pitressin. The other two patients showed a limited ability to concentrate their urine after fluid restriction sufficient to increase serum osmolality to 300 mOsm/kg or above or to lose at least 2.5% body weight.

The effects of vasopressin on cyclic AMP in urine

Table I. Clinical findings in patients with diabetes insipidus

Subject	Age, yr	Sex	Etiology	$U_{osm} \text{ Max,}^1$ mOsm/kg	Treatment
<i>DR</i>	4	M	Nephrogenic, congenital	128	Vasopressin, 2 U i.m.
<i>TR</i>	10	M	Nephrogenic, congenital	85	Vasopressin, 3 U i.m.
<i>RM</i>	8	M	Nephrogenic, acquired	82	Vasopressin, 0.16 U i.v.
<i>MD</i>	23	M	Head injury	614	Dehydration
<i>RS</i>	48	M	Posthypophysectomy and pituitary irradiation for chromophobe adenoma	377	Dehydration
<i>LB</i>	52	M	Postcryohypophysectomy for acromegaly	915	Dehydration, 18 hr, serum osmolality 293 osm/kg, plasma AVP 1.3 pg/ml

¹ $U_{osm} \text{ Max}$: maximal urine osmolality after dehydration or vasopressin; AVP: arginine vasopressin.

Fig. 1. Effects of vasopressin on serum and urine osmolality, inulin clearance (C_{In}), adenosine 3',5'-monophosphate in urine (CYCLIC AMP), urine volume (V), free water clearance (C_{H_2O}), and osmolal clearance (C_{osm}), in a normal subject, JG, male, age 23 (a), and in a patient with nephrogenic diabetes insipidus, DR, male, age 4 (b).

and renal concentration were studied. The dose was 10 mU/min for 1 hr in the normal adults, one mU/min in the patient with diabetes insipidus, and 8 mU/kg/hr for 1 hr in the children. Vasopressin produced increases in both cyclic AMP in urine and urine osmolality in JG, a normal subject, and did not increase inulin clearance (Fig. 1a, Table II). As summarized in Table II, vasopressin, 1 or 10 mU/min for 1 hr, gave significant mean increases in cyclic AMP in urine and in urine osmolality in the other normal subjects and in the patient with vasopressin-deficient diabetes insipidus. These increases were noted during the administration of vasopressin but were more pronounced in the interval after vasopressin had been stopped. Except for the study in subject JG, which had only two

periods after vasopressin (Fig. 1a), the increases in cyclic AMP in urine after treatment were statistically significant as compared with the initial control period in each of the studies in the normal subjects and in patient LB with diabetes insipidus.

Vasopressin did not change cyclic AMP in urine or urine osmolality in patient DR with nephrogenic diabetes insipidus (Fig. 1b, Table II). As summarized in Table II, vasopressin, 8 mU/kg/min for 1 hr, did not influence cyclic AMP in urine or urine osmolality in any of the patients with nephrogenic diabetes insipidus.

The results of the effects of vasopressin on concentration of cyclic AMP in urine in patients and normal subjects are summarized in Fig. 2. It can be seen that

Table II. Effects of vasopressin on the clearance of inulin (C_{In}), urine volume (V), free water clearance (C_{H_2O}), urine osmolality (U_{osm}), and adenosine 3',5'-monophosphate in urine (U_{CAMP})

	15-Min periods	C_{In} , ml/min	V , ml/min	C_{H_2O} , ml/min	U_{osm} , mOsm/kg	U_{CAMP} nmoles/min
Normal subjects						
<i>PB</i>						
	4	92 ± 9	14.4 ± 0.5	12.6 ± 0.5	39 ± 2	1.8 ± 0.2
Vasopressin	4	92 ± 11	2.8 ± 2.2 ¹	1.4 ± 2.2 ¹	562 ± 174 ²	4.6 ± 1.8 ²
	3	96 ± 8	0.8 ± 0.1 ³	-1.0 ± 0.2 ³	800 ± 14 ³	6.8 ± 1.4 ²
<i>JG</i>						
	4	104 ± 2	4.8 ± 0.3	2.4 ± 0.5	119 ± 3	2.9 ± 0.2
Vasopressin	4	90 ± 10	1.1 ± 0.5 ³	0.2 ± 0.5 ²	492 ± 122 ²	4.6 ± 0.5
	2	120 ± 10	0.6 ± 0.1 ³	-1.0 ± 0.2 ³	720 ± 23 ³	8.9 ± 0.4
<i>RG</i>						
	4	135 ± 2	7.0 ± 0.4	4.5 ± 0.4	104 ± 7	2.9 ± 0.2
Vasopressin	4	119 ± 6 ¹	1.2 ± 0.4 ³	-0.6 ± 0.6 ³	582 ± 128 ²	4.6 ± 0.5 ²
	4	132 ± 7	0.7 ± 0.7 ³	-1.3 ± 0.1 ³	804 ± 18 ³	6.4 ± 0.4 ³
<i>HN</i>						
	4	78 ± 2	11.9 ± 0.4	9.6 ± 0.4	66 ± 2	1.7 ± 0.1
Vasopressin	4	80 ± 8	1.7 ± 0.8 ³	-0.6 ± 0.9 ³	611 ± 158 ²	3.3 ± 1.0
	4	80 ± 1	0.8 ± 0.1 ²	-1.8 ± 0.1 ³	996 ± 31 ³	5.0 ± 0.4 ³
Diabetes insipidus						
<i>LB</i>						
	3	91 ± 1	12.4 ± 0.4	9.4 ± 0.4	72 ± 4	3.1 ± 0.1
Vasopressin	4	60 ± 5 ¹	2.8 ± 1.7 ¹	1.9 ± 1.2 ¹	419 ± 137 ²	3.6 ± 0.4
	4	68 ± 7 ²	1.6 ± 0.5 ³	-1.0 ± 0.3 ³	568 ± 100 ¹	4.7 ± 0.3 ¹
Nephrogenic diabetes insipidus						
<i>TR</i>						
	4	74 ± 2	10.2 ± 0.5	8.2 ± 0.1	68 ± 3	1.5 ± 0.1
Vasopressin	4	78 ± 5	9.8 ± 0.7	7.6 ± 0.6	68 ± 1	1.5 ± 0.2
	4	71 ± 2	8.4 ± 0.3 ¹	6.6 ± 0.3 ¹	64 ± 1	1.6 ± 0.1
<i>DR</i>						
	4	28 ± 1	4.1 ± 0.1	3.4 ± 0.1	47 ± 2	0.7 ± 0.1
Vasopressin	4	30 ± 1	4.5 ± 0.2	3.8 ± 0.1	44 ± 1	0.7 ± 0.1
	4	30 ± 1	4.1 ± 0.1	3.4 ± 0.1	51 ± 1	0.7 ± 0.1
<i>RM</i>						
	3	67 ± 4	5.2 ± 0.2	3.8 ± 0.1	76 ± 1	1.4 ± 0.2
Vasopressin	4	68 ± 2	5.2 ± 0.2	3.8 ± 0.1	76 ± 2	1.6 ± 0.1
	4	65 ± 1	5.2 ± 0.2	3.9 ± 0.2	68 ± 3	1.6 ± 0.1

¹ $P < 0.01$ as compared with initial control value. Results are given as mean ± 1 SE.

² $P < 0.05$.

³ $P < 0.001$.

with vasopressin there were 10-fold or greater increases in the concentration of the cyclic nucleotide in each of the four normal subjects and in the patient with diabetes insipidus, but no change in the three patients with nephrogenic diabetes insipidus.

The effects of cyclic AMP (0.2–0.6 mg/kg/min) on inulin clearance, free water clearance, urine volume, and urine osmolality were examined (Table III). Cyclic AMP did not change any of these in two of the patients with nephrogenic diabetes insipidus. Cyclic AMP increased urine osmolality and lowered free water clearance and urine volume in each of the three patients with vasopressin-deficient diabetes insipidus

and in the third patient with nephrogenic diabetes insipidus. However, there was a modest decrease in inulin clearance in each of the four patients and it cannot be certain that the increases in urine concentration did not result from the decrease in glomerular filtration rate [3].

Discussion

There is convincing evidence that the effects of vasopressin on the kidney to produce a concentrated urine are mediated through the adenylate cyclase system. Vasopressin has been shown to activate adenylate cy-

clase in the medullary portion of rat kidney [5], to increase the concentration of cyclic AMP in homogenates of dog kidney [4], and to increase the excretion of cyclic AMP in urine in man [9, 18]. Vasopressin and cyclic AMP apparently influence renal concentration by increasing the permeability of collecting tubules to water [12].

The present results show that patients with nephrogenic diabetes insipidus are unable to increase the rate of excretion of cyclic AMP after vasopressin. In contrast, vasopressin produced significant increases in the rate of excretion of the cyclic nucleotide in the normal subjects and in the patient with diabetes insipidus. Similar findings have also been reported independently by Fichman and Brooker [9]. Taken together these results support the view that there is a defect in the adenylate cyclase system in nephrogenic diabetes insipidus which accounts for the lack of response to vasopressin. It should be noted that patients with other diseases associated with impaired renal concentrating ability, potassium deficiency or sickle cell anemia, have been shown to increase urinary cyclic AMP in response to vasopressin [16].

It is not possible on the basis of the information now available to determine whether the lack of increase in cyclic AMP in urine in response to vasopressin in nephrogenic diabetes insipidus results from failure of the receptors to bind the hormone, an abnormality in the enzyme itself, or both. Also, it is not known whether the abnormality is limited to the adenylate cyclase system or if there is an overall defect which includes the response mechanism to cyclic AMP as well. We sought unsuccessfully to answer this question by comparing the effects of cyclic AMP on renal concentrating ability in patients with nephrogenic diabetes insipidus and patients with vasopressin-deficient diabetes insipidus. As noted in the results, cyclic AMP did not produce increases in urine osmolality in two of the three patients with nephrogenic diabetes insipidus. Jones and associates [13] also were unable to show an increase in urine osmolality with cyclic AMP in three patients with nephrogenic diabetes insipidus even though urine volume decreased in two of them. Levine [15] noted decreases in urine volume and increases in urine osmolality in patients with diabetes insipidus given cyclic AMP. However, glomerular filtration rate was not measured in his studies and again it cannot be determined whether the observed changes were produced by alteration in glomerular filtration rate. In the present studies, there were modest increases in urine osmolality in the three patients with vasopressin

Fig. 2. Effects of vasopressin on urine concentration of adenosine 3',5'-monophosphate (Cyclic 3',5'-AMP) in four normal subjects, one patient with diabetes insipidus (DI), and three patients with nephrogenic diabetes insipidus (Neph. DI).

deficiency and in the third patient with nephrogenic diabetes insipidus, but these changes were always associated with decreases in the clearance of inulin. Consequently, it was never possible to determine whether the observed changes in urine osmolality resulted from an effect of cyclic AMP to increase the permeability of the collecting duct or from decreases in glomerular filtration [3].

Pseudohypoparathyroidism is a disease in which parathyroid hormone unresponsiveness has been related to an abnormality in the formation of cyclic AMP [6]. We have been able to demonstrate that the defect is probably limited to the adenylate cyclase system by showing a normal response to dibutyryl cyclic AMP in several such patients [1].

Finally, it is worthwhile to emphasize that the changes in concentration of cyclic AMP in urine were far more striking than the changes in rate of excretion of the cyclic nucleotide in response to vasopressin in the normal subjects and patient LR with diabetes insipidus. Because there is probably a dynamic state of equilibrium of cyclic AMP between the renal tubular cells and the urine, a 10-fold decrease in urine volume after vasopressin would require a 20- to 40-fold increase in the concentration of cyclic AMP in vasopres-

Table III. Effects of adenosine 3',5'-monophosphate (CAMP) on the clearance of inulin (C_{In}), urine volume (V), free water clearance (C_{H_2O}), and urine osmolality (U_{osm})

Patient	Period	Duration, min	CAMP, mg/kg/min	C_{In} , ml/min	V , ml/min	C_{H_2O} , ml/min	U_{osm} , mOsm/kg
Nephrogenic diabetes insipidus							
<i>TR</i>							
	1	0-20		53	10.3	8.0	65
	2	20-40		49	9.8	7.6	62
	3	40-55		45	9.3	7.4	61
	4	55-70		45	9.3	7.4	61
	5	70-85	0.2	45	10.4	8.4	57
	6	85-100	0.2	40	11.3	9.2	54
	7	100-115	0.4	40	10.5	8.7	51
	8	115-130	0.4	42	10.9	9.2	48
	9	130-145	0.6	42	11.6	9.7	48
	10	145-160	0.6	43	11.2	9.4	48
	11	160-175		46	9.1	7.7	48
<i>DR</i>							
	1	0-15		32	4.3	3.7	39
	2	15-30		44	4.2	3.6	45
	3	30-46		40	3.9	3.2	49
	4	46-60	0.2	31	3.9	3.2	49
	5	60-75	0.2	38	3.7	3.1	47
	6	75-90	0.4	36	3.3	2.8	44
	7	90-121	0.4	21	3.0	2.4	55
	8	121-158	0.4	21	3.0	2.5	47
	9	158-172	0.4	21	3.0	2.6	40
<i>RM</i>							
	1	0-14		81	4.7	3.0	101
	2	14-28		82	4.9	3.4	83
	3	28-44		70	4.9	3.6	70
	4	44-59		67	5.2	3.9	67
	5	59-74	0.2	63	5.2	4.1	60
	6	74-88	0.2	64	5.1	4.1	52
	7	88-104	0.4	53	2.5	1.8	76
	8	104-119	0.4	50	1.3	0.7	118
	9	119-133	0.6	61	2.1	1.4	91
	10	133-148	0.6	54	2.1	1.5	81
	11	148-177		63	4.3	3.5	51
	12	162-177		70	6.9	6.0	37
Diabetes insipidus							
<i>RS</i>							
	1	0-16		219	5.5	5.6	116
	2	16-30		167	6.1	4.8	74
	3	30-45		182	7.1	4.9	103
	4	45-60		165	6.5	4.3	107
	5	60-76	0.3	144	5.9	4.2	88
	6	76-118	0.3	122	1.5	0.8	143
	7	118-136		122	1.3	0.2	238
	8	136-150		106	2.1	0.2	233
	9	148-164		106	2.4	0.7	185
<i>LB</i>							
	1	0-15		104	7.1	3.2	158
	2	15-30		114	7.7	4.4	125
	3	30-45		104	9.3	5.4	122
	4	45-60		104	8.6	5.1	121
	5	60-75	0.3	109	10.5	6.2	120
	6	75-90	0.3	118	9.6	5.6	122
	7	90-105	0.4	96	8.6	5.1	119
	8	105-127	0.4	79	5.0	2.4	152
	9	127-150		77	2.0	-0.1	308
	10	150-166		77	4.1	0.7	242
	11	166-183		88	8.1	4.0	147

Table III.—Continued

Patient	Period	Duration, min	CAMP, mg/kg/min	C _{In} , ml/min	V, ml/min	CH ₂ O, ml/min	U _{osm} , mOsm/kg
Diabetes insipidus (<i>cont.</i>)							
<i>MD</i>							
	1	0-15		176	6.6	3.6	128
	2	15-33		120	6.6	4.3	101
	3	33-48		144	8.9	6.0	93
	4	48-63		126	7.2	4.8	97
	5	63-79		126	7.2	4.8	86
	6	79-150	0.3	103	2.3	0.8	89
	7	150-169		105	0.6	-0.1	189
	8	169-183		105	2.9	-0.8	363
	9	183-198		115	2.4	0.1	369

sin-responsive cells to effect a 2- to 4-fold increase in the rate of excretion of cyclic AMP in urine. This could account for the rather modest increases in cyclic AMP in urine in response to vasopressin observed in the present and previous studies [6, 9, 18] as compared with parathyroid hormone, for example [14], and could explain the inability in some laboratories to demonstrate significant increases in the rate of excretion of cyclic AMP in urine with vasopressin [6, 14].

Summary

To determine whether a defect in the adenylate cyclase system might be responsible for the lack of response to vasopressin in patients with nephrogenic diabetes insipidus, the effects of vasopressin on cyclic AMP in urine and renal concentration were examined by standard clearance techniques. Studies were carried out in three patients with vasopressin-resistant diabetes insipidus, in one patient with diabetes insipidus due to vasopressin deficiency, and in four normal subjects. Serum and urine osmolality, inulin clearance, and cyclic AMP in urine were determined. In the normal subjects and in the patient with vasopressin deficiency, vasopressin produced significant mean increases in cyclic AMP in urine and caused renal concentration. In contrast, vasopressin did not influence either cyclic AMP in urine or renal concentration in the patients with nephrogenic diabetes insipidus. Studies of the effects of cyclic AMP were also determined in the three patients with nephrogenic diabetes insipidus and in three patients with vasopressin deficiency. The studies were inconclusive and it could not be certain that increases in urine concentration with cyclic AMP were produced by decreases in glomerular filtration rate. These results support the hypothesis that a defect in the adenylate cyclase system may be responsible for

the impairment in concentrating ability in vasopressin-resistant diabetes insipidus.

References and Notes

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25. Requests for reprints should be addressed to: N. H. BELL, M.D., Veterans Administration Hospital, 1481 W. 10th St., Indianapolis, Ind. 46202 (USA).
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