The Effect of Exogenous 3':5'-Adenosine Monophosphate on Urinary Output in Children with Vasopressin-Resistant Diabetes Insipidus

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Extract

3':5'-Adenosine monophosphate (c-AMP) and dibutyl 3':5'-adenosine monophosphate (DB c-AMP) were given intravenously to six children with nephrogenic diabetes insipidus. Both nucleotides were unable to reduce, in a constant and reproducible manner, urine volume and free water clearance. Paradoxically, DB c-AMP, given as an infusion, caused a clear-cut rise in urine volume (an increase of 138.0—188.5%, mean of 172.8%, as compared with the preceding periods) without influencing creatinine excretion.

Speculation

In nephrogenic diabetes insipidus, the basic defect responsible for vasopressin resistance presumably is located beyond the intracellular c-AMP formation. DB c-AMP might diminish the net reabsorption of water and solutes in the proximal renal tubule.

c-AMP is the second messenger of many hormones including the antidiuretic hormone (ADH) (15). Therefore, the administration of exogenous c-AMP mimics the action of the hormone, causing antidiuresis in normal and vasopressin-deficient subjects (11).

The present investigation was undertaken to study the effect of intravenously injected c-AMP and of its dibutyl derivative (DB c-AMP) on diuresis (V), urine osmolality (U_{osm}), osmolar clearance (C_{osm}), and free water clearance (C_{H_2O}) in six patients with nephrogenic diabetes insipidus (NDI).

METHODS, PATIENTS, AND SPECIAL STUDIES

METHODS

All children were studied after informed consent of the parents had been obtained. An intravenous infusion with 5% glucose or 0.9% sodium chloride solution was maintained throughout each test at a rate of 0.5–2 ml/min. The volume of infusate expressed in milliliters per minute per square meter varied between 1.35 and 1.88 in the individual patients with a mean of 1.58. Urine was obtained in 15-min portions by an indwelling catheter. Plasma samples for measurement of osmolality (P_{osm}) were taken every hour. The patients were not fasted and during the test they were offered water hourly in amounts equal to the urinary output in the preceding clearance period. They regularly took less than these amounts and on the whole, the individual water balances remained unchanged up to the start of the last clearance period. The U_{osm} was measured as well as its volume which was expressed in milliliters per minute (V). C_{osm} and C_{H_2O} were calculated according to the equations: $C_{osm} = (U_{osm} \times V)/P_{osm}$ and $C_{H_2O} = V - C_{osm}$. A Knauer osmometer was used to determine P_{osm} and U_{osm} . Blood

glucose was measured by the glucose oxidase method, plasma nonesterified free fatty acids (NEFA) by a modification of the method of Dole (6), and plasma growth hormone (GH) by radioimmunoassay with the use of the double antibody method (16).

3':5'-Adenosine monophosphate and N⁶-2'-O-dibutyl adenosine 3':5'-monophosphate monosodium salts were purchased from Sigma and prepared for human use as follows: the dry powder was dissolved in a 0.9% solution of NaCl to a concentration of 5 mg/ml and adjusted to pH 7.4 with 0.01 N of NaOH. The solutions were sterilized by filtration on Millipore filters. L₈-vasopressin was purchased from Sandoz and diluted in 0.9% NaCl solution. The antidiuretic activity of the c-AMP preparation was established by a single injection in one 10-month-old control patient, a mentally retarded, physically healthy infant without any abnormality in renal function. The biologic activity of both the c-AMP and DB c-AMP preparation was shown by their effects on blood glucose, plasma growth hormone, and NEFA in one patient.

PATIENTS

The six patients have nephrogenic diabetes insipidus (Table 1). The first two are unrelated girls. The third patient, a boy, is the child of a consanguineous marriage between cousins. The three other cases are brothers. All six children showed no other abnormality than their concentration inability which was uninfluenced by vasopressin. Their glomerular filtration, estimated by endogenous creatinine clearance, was normal.

SPECIAL STUDIES

AC, as the first patient with NDI to be submitted to our c-AMP and DB c-AMP investigations, was studied in more detail.

Protocol I. Alternating intravenous injections of c-AMP and pitressin at hourly intervals.

In *patient AC*, after a control period of 1 hr, 6 mg/kg body wt c-AMP was injected slowly. One hour later, 2 units of pitressin were given by i.v. injection. After the same interval of 1 hr, c-AMP injection was repeated at a dose of 10 mg/kg, followed by 1 unit pitressin. In the five other patients with NDI, pitressin was injected only once and at a dose of 0.1 unit.

Protocol II. Infusion of c-AMP and DB c-AMP. After a control period of 45–60 min, 0.5 mg/kg/min c-AMP was perfused during 45–60 min, followed by an infusion of 0.2 mg/kg/min of DB c-AMP during the same period of time. Both periods were separated by a wash-out time of 30 min. This experiment was done in the six NDI patients. In *patient AC*, blood glucose, NEFA, and GH were followed at 15-min intervals.

Protocol III. Injection of DB c-AMP, Aminophyllin, and Association of c-AMP and Aminophyllin. After a 30-min control

period, 5 mg/kg DB c-AMP and 3 mg/kg aminophyllin were injected consecutively at 1-hr intervals. After the same interval, both c-AMP, 15 mg/kg, and aminophyllin, 3 mg/kg, were injected simultaneously. This protocol was applied only to *patient AC*.

RESULTS

PROTOCOL I

The results of *protocol I* are shown in Figure 1 and in Table 2. There is no indication of any clear-cut antidiuretic effect either by the two doses of c-AMP, or by pitressin. On the contrary, c-AMP at a dose of 10 mg/kg is associated with a rise in urinary volume in four patients out of the six. Throughout the successive test periods, there are only minor variations in creatinine excretion.

PROTOCOL II

The results obtained by applying *protocol II* are shown in Figure 2 and in Table 3.

The biological activity of the infused cyclic nucleotides is shown in *patient AC* (Fig. 2) by the changes in serum NEFA, plasma GH, and blood glucose known to occur during the administration of c-AMP and DB c-AMP (12, 13). An antidiuresis is only observed in *patient MF* and to a lesser degree in *patient AC* under the influence of c-AMP, but is not reproduced during DB c-AMP infusion. In the other patients, the effect of c-AMP on urinary volume is negligible. The infusion with DB c-AMP, on the other

Table 1. Clinical data for children with nephrogenic diabetes insipidus¹

Pa- tients	Sex	Age		Urine sediment and culture	Protein- uria, gluco- suria	I.V.P.
		Diag- nosis	Study			
AC	F	5 mo	6 mo	Neg	Neg	nl
NS	F	3 mo	6 mo	Neg	Neg	nl
MF	M	6 mo	8 mo	Neg	Neg	nl
CE	M	6 wks	3 yr	Neg	Neg	nl
LE	M	18 mo	8 yr	Neg	Neg	nl
FE	M	3 yrs	9 yr	Neg	Neg	nl

¹ Neg: negative; I.V.P.: intravenous pyelography; nl: normal.

Fig. 1. The effect of alternating administration of 3':5'-adenosine monophosphate (cAMP) and pitressin in two different dosages each on urinary volume, urinary osmolality, osmolar clearance, and free water clearance in *patient AC* with nephrogenic diabetes insipidus (*protocol I*).

Table 2. Results of alternating injection of 3':5'-adenosine monophosphate (c-AMP) and pitressin in six patients with nephrogenic diabetes insipidus¹

	Control period	c-AMP, 6 mg/kg	Pitressin	c-AMP, 10 mg/kg
AC				
V	1.18	1.36	1.37	2.12
U _{osm}	79	36	53	34
C _{osm}	0.30	0.15	0.21	0.23
C _{H₂O}	0.88	1.21	1.16	1.89
Creatinine	0.30	0.25	0.22	0.30
NS				
V	1.10	0.84	0.94	1.20
U _{osm}	180	134	172	120
C _{osm}	0.61	0.36	0.46	0.44
C _{H₂O}	0.49	0.48	0.48	0.76
Creatinine	0.28	0.19	0.26	0.21
MF				
V	1.58	1.66	1.43	2.03
U _{osm}	98	61	86	52
C _{osm}	0.44	0.40	0.43	0.36
C _{H₂O}	1.14	1.26	1.00	1.67
Creatinine	0.32	0.29	0.29	0.31
CE				
V	2.45	2.43	3.50	3.79
U _{osm}	71	63	48	42
C _{osm}	0.56	0.50	0.56	0.52
C _{H₂O}	1.89	1.93	2.94	3.27
Creatinine	0.53	0.53	0.61	0.58
LE				
V	7.65	6.87	7.31	5.23
U _{osm}	78	59	50	54
C _{osm}	2.20	1.41	1.36	1.08
C _{H₂O}	5.45	5.46	5.95	4.15
Creatinine	0.91	0.73	0.73	0.70
FE				
V	7.36	6.42	6.89	13.83
U _{osm}	67	65	59	70
C _{osm}	1.71	1.38	1.41	3.69
C _{H₂O}	5.65	5.04	5.48	10.14
Creatinine	0.85	0.75	0.93	0.98

¹ The figures are mean values of urine volume (V) and osmolality (U_{osm}), osmolar clearance (C_{osm}), free water clearance (C_{H₂O}), and creatinine excretion for each period. V, C_{osm}, and C_{H₂O} are expressed as milliliters per minute, U_{osm} as milliosmolar concentration per kilogram H₂O and creatinine excretion as milligrams per minute and per 1.73 m².

hand, results in a rise of urinary volume in all patients. As during *protocol I*, the creatinine excretions remain unchanged.

PROTOCOL III

The results are presented in Table 4.

There was no antidiuresis during the whole procedure. On the contrary, urinary volumes rose steadily together with C_{osm} and C_{H₂O}. Creatinine excretion remained unchanged.

DISCUSSION

c-AMP is the intracellular mediator of the ADH or vasopressin. The role of c-AMP as the second messenger of ADH has been demonstrated by the following experiments: (1) c-AMP changes the permeability to water in the isolated toad bladder in the same way as does vasopressin (14); (2) vasopressin increases the intracellular concentration of c-AMP in the toad bladder (3, 9); (3) vasopressin activates adenylcyclase in the rat renal medulla (4); (4) exogenous c-AMP, given to normal and vasopressin-deficient

Fig. 2. Values of urinary volume, osmolar clearance, and free water clearance, blood glucose, plasma nonesterified fatty acids and growth hormone levels in patient AC before and during 3':5'-adenosine monophosphate (cAMP) or dibutyryl 3':5'-adenosine monophosphate (DBc-AMP) (protocol II).

subjects, reduces V and C_{H_2O} in a clear-cut fashion (11). This finding was confirmed in our control patients as shown in Figure 3. The exact mechanism by which c-AMP changes the permeability of the tubular membrane is unknown. It has been assumed (7) that c-AMP activates a specific protein kinase. This in turn catalyzes the phosphorylation of a membrane protein, bringing about a change in the structure of the membrane and enhancing its permeability to water. Accordingly, the effectuation of normal vasopressin activity would involve a chain of biochemical processes. It seems useful to subdivide the overall mechanism into two major steps: (1) hormone-stimulated formation of c-AMP and (2) c-AMP-initiated increase of membrane permeability to water. NDI is a so-called pseudo-endocrine disorder, i.e., a disease in which there is no hormone deficit but an end-organ unresponsiveness to the hormone, in this case ADH. Theoretically, NDI could result from a defect in either of both steps of vasopressin action. A defect in the first step could result either from an anomaly of the cellular membrane receptor or from the inactivity of adenylylase. In both cases, one would expect exogenous c-AMP to promote antidiuresis in this disease. Our studies are clearly not in favor of this first hypothesis.

In our six patients with NDI no reproducible reduction of V and C_{H_2O} was obtained either after pitressin or c-AMP and DB c-AMP administration. Our findings confirm those of Avery *et al.* (1). These authors reported briefly on the effect of c-AMP and DB c-AMP by constant infusion to patients with NDI. They did not observe any change in C_{H_2O} , U_{osm} , and glomerular filtration rate during c-AMP infusion. In contrast, DB c-AMP increased C_{H_2O} . We found the same seemingly paradoxical effect of DB c-AMP together with a definite rise in the urinary volumes in our six patients. At the dosages used, especially in protocol II, the differences between c-AMP and DB c-AMP are unmistakable. The effect of DB c-AMP cannot be attributed to changes in the glomerular filtration rate, since the creatinine excretion remains unchanged during the several test periods. Neither did overhydration play a role: the patients' water balances were roughly in equilibrium at the beginning of the DB c-AMP infusion. There-

Table 3. Results of 3':5'-adenosine monophosphate (c-AMP) and dibutyryl 3':5'-adenosine monophosphate (DBc-AMP) perfusions in six patients with nephrogenic diabetes insipidus¹

	Control period	c-AMP, 0.5 mg/kg · min	Wash-out	DB c-AMP, 0.2 mg/kg · min
AC				
V	2.90	2.03	3.29	4.82
U_{osm}	40	37	36	47
C_{osm}	0.38	0.25	0.41	0.78
C_{H_2O}	2.52	1.78	2.88	4.04
Creatinine	0.35	0.32	0.34	0.34
NS				
V	0.89	1.08	0.86	1.69
U_{osm}	147	114	163	105
C_{osm}	0.44	0.41	0.47	0.62
C_{H_2O}	0.45	0.67	0.39	1.07
Creatinine	0.26	0.23	0.23	0.22
MF				
V	1.53	0.68	1.65	2.23
U_{osm}	61	111	104	54
C_{osm}	0.29	0.26	0.58	0.41
C_{H_2O}	1.24	0.42	1.07	1.82
Creatinine	0.25	0.21	0.28	0.25
CE				
V	3.58	3.53	3.95	5.09
U_{osm}	92	53	44	45
C_{osm}	1.10	0.66	0.60	0.79
C_{H_2O}	2.48	2.87	3.35	4.30
Creatinine	0.44	0.40	0.44	0.42
LE				
V	5.89	5.22	7.21	11.51
U_{osm}	136	116	88	83
C_{osm}	2.75	2.15	2.12	3.40
C_{H_2O}	3.14	3.07	5.09	8.11
Creatinine	0.76	0.70	0.72	0.73
FE				
V	8.43	7.58	6.63	13.76
U_{osm}	78	80	75	87
C_{H_2O}	2.45	2.19	1.62	4.22
C_{H_2O}	5.98	5.39	5.01	9.54
Creatinine	0.76	0.81	0.77	0.79*

¹ For explanation of data, see footnote 1 of Table 2.

Fig. 3. The effect of 3':5'-adenosine monophosphate (c-AMP) and pitressin on urinary volume, urinary osmolality, osmolar clearance, and free water clearance in a normal control child.

Table 4. Results of alternating injection of dibutyryl 3':5'-adenosine monophosphate (DB c-AMP), aminophyllin, and 3':5'-adenosine monophosphate (c-AMP) combined with aminophyllin in patient AC with nephrogenic diabetes insipidus¹

	Control period	DB c-AMP, 5 mg/kg	Amino-phyllin, 3 mg/kg	c-AMP, 15 mg/kg, Amino-phyllin
V	2.52	2.91	4.16	5.23
U _{osm}	34	32	35	32
C _{osm}	0.28	0.30	0.48	0.72
C _{H₂O}	2.24	2.60	3.68	4.51
Creatinine	0.36	0.35	0.34	0.35

¹ For explanation of data, see footnote 1 of Table 2.

fore, one might postulate that at least in patients with NDI, DB c-AMP diminishes the water reabsorption at the level of the proximal renal tubule. Such an effect would indeed account for the observed changes in V, C_{H₂O}, and C_{osm}. It is noteworthy that a diuretic effect combined with stable creatinine excretion was also the salient feature observed in *protocol III*. This indicates that in NDI patients aminophyllin does not restore the antidiuretic effect of c-AMP observed in normal individuals.

Jones *et al.* (10) found that c-AMP transiently reduced urinary output in two of three patients with NDI. A similar effect was noted but only irregularly in two of our six patients. It was, however, rather mild, so that the changes observed could have been spontaneous ones. Avery's and our results would indicate that the pathogenesis of NDI is located beyond c-AMP formation and consists of a defect in the second step of vasopressin action. However, experiments of Fichman and Brooker (8) are at variance with this "second step hypothesis." These authors observed a deficient urinary c-AMP excretion after infusion of pitressin in NDI patients. They concluded therefore that the pathogenesis of NDI is analogous to that of pseudohypoparathyroidism (PHP). PHP is indeed an example of a pseudoendocrine disorder in which a first step defect is likely to be present. In patients with PHP, Chase *et al.* (5) showed a defective excretion of c-AMP in response to parathyroid hormone administration, whereas Bell *et al.* (2) found that DB c-AMP can reproduce the effect of parathyroid hormone. In contrast to the apparently clear-cut situation in PHP, the deficient renal c-AMP excretion under influence of exogenous ADH on one hand and the inactivity of c-AMP infusions on the other hand is paradoxical in NDI. Concerning these apparently contradictory facts, three remarks require consideration. First, the lack of urinary increase of c-AMP after pitressin administration could be the result of a defective release from the collecting duct cells. This possibility, among others, is put forward by Fichman and Brooker (8). It would be in keeping with the knowledge that, whatever its pathogenesis, NDI is characterized by a disturbance in the permeability of the cell membrane on the tubular luminal side, the membrane through which intracellular c-AMP is thought to leak into the urine. The second point is that exogenous c-AMP could be unable to enter the target cells and mimic the effect of the hormone. In that respect, it may be pointed out that, in our studies, c-AMP and DB c-AMP were used in relatively high doses and

given both as a single shot and in perfusion. Last, it cannot be excluded that in NDI the intracellular breakdown of c-AMP in the renal tubular cells is so high that the cyclic nucleotide cannot exert its normal activity. This could also explain the low values of urinary c-AMP excretion found to occur in NDI patients after parathyroid hormone administration (8).

SUMMARY

The administration of c-AMP and DB c-AMP to six children with NDI has failed to yield an antidiuretic effect. From the present study it may be concluded that, at the doses used, neither c-AMP nor its dibutyryl derivative can mimic the action of ADH in NDI as they do in normal subjects. On the contrary, DB c-AMP increases urine volume and C_{osm} in a very marked way, without changing the creatinine excretion.

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