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Effects of adenosine 3′,5′-monophosphate on renal function in the rabbit

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Summary

1. Intravenous injections of adenosine 3′,5′-monophosphate (cyclic-AMP) were given to five conscious, diuretic rabbits, and the effects on urinary flow and composition were compared with those of intravenous injections of vasopressin given to the same animals.

2. Cyclic-AMP produced antidiuresis with increases in urinary osmolality and in the urinary concentrations of creatinine, urea and usually of sodium.

3. The fractional reabsorption of urea from renal tubular fluid increased during cyclic-AMP induced antidiuresis.

4. The effects of cyclic-AMP thus closely resembled those of vasopressin and are consistent with the view that cyclic-AMP mediates the action of this hormone.

Introduction

Orloff & Handler (1962, 1964, 1967) have presented evidence that vasopressin acts on responsive tissues by increasing the rate of formation of adenosine 3′,5′-monophosphate (cyclic-AMP), and that this nucleotide causes the changes in membrane permeability which characterize the effect of vasopressin. Thus cyclic-AMP closely mimics the action of vasopressin on the toad bladder preparation, increasing the permeability to water of this membrane and stimulating active sodium transport (Orloff & Handler, 1962 ; Edelman, Petersen & Gulyassy, 1964). Moreover, vasopressin increases the concentration of cyclic-AMP in the cells of the toad bladder (Handler, Butcher, Sutherland & Orloff, 1965). That these findings in the toad bladder are relevant to the mammalian kidney is suggested by the fact that vasopressin also stimulates production of cyclic-AMP by homogenates of dog kidney (Brown, Clarke, Roux & Sherman, 1963), and by the report that both vasopressin and cyclic-AMP increase the permeability to water of isolated rabbit collecting tubules (Grantham & Burg, 1966).

The recent observation that vasopressin *in vivo* reduces the level of adenosine triphosphate (ATP) in the inner medulla of rat kidney (Jones & Welt, 1967) suggests that the above *in vitro* studies of the metabolic effects of vasopressin are relevant to its action in the intact animal. Cyclic-AMP is present in human urine (Butcher & Sutherland, 1962) and it has been claimed that urinary excretion of this nucleotide is reduced in diabetes insipidus (Takahashi, Kamimura, Shinko & Shozo, 1966). There is, however, very little information about the effects of cyclic-AMP on renal function in the intact animal, although Levine (1967) observed that intravenous

injections of the nucleotide reduced urine flow in man. In the present study we have examined the effects of cyclic-AMP on renal function in the conscious rabbit.

Methods

The experiments were performed on five conscious rabbits, weighing between 2 and 2·5 kg, which had been habituated to the experimental conditions (Barraclough, Jones, Marsden & Bradford, 1967). Three to four days before the experiment indwelling venous and bladder catheters were inserted under anaesthesia. After this operation each animal was housed in a cage permitting freedom of movement except for backward movement of the head, which was prevented by a neck halter. The animals ate and drank freely until the day of the experiment, when food was withheld and a diuresis obtained by infusing intravenously $\frac{1}{4}$ strength Hartmann's solution at 0·75 or 1·5 ml/min by a constant infusion pump. When urine flow rate had become steady control collections were made over 10 min periods and either vasopressin (" Pitressin ", Parke Davis), in doses of 5 to 10 mu., or cyclic-AMP, in doses of 25 to 54 mg, was injected into the infusion tubing. Great care was taken not to disturb the animals and to ensure that no air bubbles were introduced with these injections. All injections were given in such a way that the total rate of fluid input was unchanged.

Endogenous creatinine was used as a " glomerular substance ", a use which has been justified for acute experiments such as these (Thomas, 1964 ; Jones *et al.*, 1967). In one study ^{57}Co-cyanocobalamin (Radiochemical Centre, Amersham) was used in addition to creatinine as a glomerular marker (Nelp, Wagner & Reba, 1964).

Urine samples were analysed for osmolality (Aminco-Bowman Osmometer), creatinine and urea (Auto-Analyzer, Technicon), and sodium (flame photometer).

Cyclic-AMP was supplied by Koch-Light Chemicals Ltd. as a powder. It was dissolved in 0·5 N NaOH, adjusted to pH 7·8 with phosphoric acid and filtered through sintered glass (porosity 5 on 3) into sterile containers.

In three experiments injections were given of the vehicle in which cyclic-AMP was dissolved.

Results

In each animal vasopressin, in doses of 5 or 10 mu., reduced urine flow and increased urinary osmolality and the urinary concentration of sodium and creatinine (Table 1).

The effects of cyclic-AMP on renal function (Table 2) closely resembled those of vasopressin: after every injection of cyclic-AMP urinary flow fell sharply, while urinary osmolality and creatinine concentration increased. In four of the five experiments urinary sodium concentration also increased after injection of cyclic-AMP (Fig. 1). No attempt was made to establish a dose-response relationship in these experiments, but in rabbit F, 30 mg of cyclic-AMP produced a similar antidiuresis to 5 mu. of vasopressin, while in rabbits A, B and C 10 mu. vasopressin had a larger antidiuretic effect than 25 or 30 mg cyclic-AMP (Tables 1 and 2). The time courses of antidiuresis produced by these two agents were similar (Fig. 2).

Injections of the vehicle used to dissolve cyclic-AMP were without effect (Fig. 2).

TABLE 1. *Effect of vasopressin on urinary solute concentrations*

Rabbit	Vasopressin dose (mu.)	Period	Time from injection (min)	Urine flow (ml/min)	Urinary concentrations				Urinary solute concentration ratios experimental/control			
					Cr. (mg/l.)	Urea (mmol/l.)	Osm. (mosmol/kg)	Na (mequiv./l.)	Cr.	Urea	Osm.	Na
A		C*		1·36	75	36	159	44	0·83	0·88	0·80	0·95
		C		1·49	90	41	198	47	1·00	1·00	1·00	1·00
	10	E+	32	0·17	940	353	700	118	10·44	8·62	3·54	2·51
B		C		1·07	82	38	151	25	1·37	1·34	1·39	1·35
		C		1·23	60	29	109	18	1·00	1·00	1·00	1·00
	10	E	54	0·16	504	211	646	61	8·40	7·69	5·93	3·34
C		C		1·58	65	33	97	24	1·00	1·00	1·00	0·92
		C		1·25	66	32	95	26	1·00	1·00	1·00	1·00
	10	E	70	0·16	464	176	468	108	7·03	5·44	4·93	4·15
F		C		0·58	86	32	66	8	1·00	1·02	1·06	1·16
		C		0·63	86	32	62	7	1·00	1·00	1·00	1·00
	5	E	56	0·35	180	54	113	10	2·09	1·71	1·82	1·54
G		C		2·18	45	18	124	32	1·00	1·00	0·95	0·87
		C		2·32	45	18	130	37	1·00	1·00	1·00	1·00
	10	E	15	0·50	135	44	313	78	3·00	2·52	2·41	2·11

C* indicates the two control periods immediately before injection of vasopressin, and E+ refers to the period during the ensuing antidiuresis in which urinary creatinine (Cr.) concentration was highest. Osm, Osmolality.

TABLE 2. *Effect of cyclic-AMP on urinary solute concentrations*

Rabbit	Cyclic-AMP dose (mg)	Period	Time from injection (min)	Urine flow (ml/min)	Urinary concentrations				Urinary solute concentration ratios experimental/control			
					Cr. (mg/l.)	Urea (mmol/l.)	Osm. (mosmol/l.)	Na (mequiv./l.)	Cr.	Urea	Osm.	Na
A		C*		1·70	58	31	139	22	0·97	1·00	1·13	0·82
		C		1·58	60	31	123	27	1·00	1·00	1·00	1·00
	25	E+	33	0·43	230	93	435	73	3·83	3·04	3·54	2·67
B		C		1·10	61	25	133	29	0·82	0·85	0·92	0·94
		C		1·02	74	33	144	31	1·00	1·00	1·00	1·00
	25	E	43	0·47	149	59	208	43	2·01	1·76	1·44	1·39
C		C		1·57	60	32	103	25	1·15	1·16	1·13	1·04
		C		1·53	52	27	91	24	1·00	1·00	1·00	1·00
	25	E	20	0·38	142	57	173	59	2·73	2·07	1·90	2·46
F		C		0·61	90	38	73	7	1·05	1·10	1·00	0·90
		C		0·61	86	34	73	7	1·00	1·00	1·00	1·00
	30	E	46	0·22	180	58	132	10	2·09	1·69	1·81	1·43
G		C		1·88	49	21	126	34	0·98	1·00	1·00	0·71
		C		1·88	50	21	126	47	1·00	1·00	1·00	1·00
	54	E	33	0·18	148	35	218	44	2·96	1·66	1·73	0·92

C* and E+ refer to control and antidiuretic periods as defined in Table 1. Cr., Creatinine; Osm., osmolality.

Urea excretion

The effect of vasopressin on urea excretion (see above) is shown by expressing the results as " solute concentration ratios ", which are derived for each solute by dividing its concentration in every urine sample by its concentration in the control diuretic period immediately before injection of vasopressin or of cyclic-AMP. Table 1 and Fig. 3 show that the concentration ratio for urea rose less than that for creatinine after vasopressin. Table 2 and Fig. 3 show that the concentration ratio for urea also rose less than that for creatinine after cyclic-AMP and the quantitative relationship between these variables following injection of cyclic-AMP was similar to that seen after vasopressin (Fig. 4).

Discussion

The results show that intravenous injection of cyclic-AMP produces an anti-diuresis in which changes in urinary composition closely resemble those produced

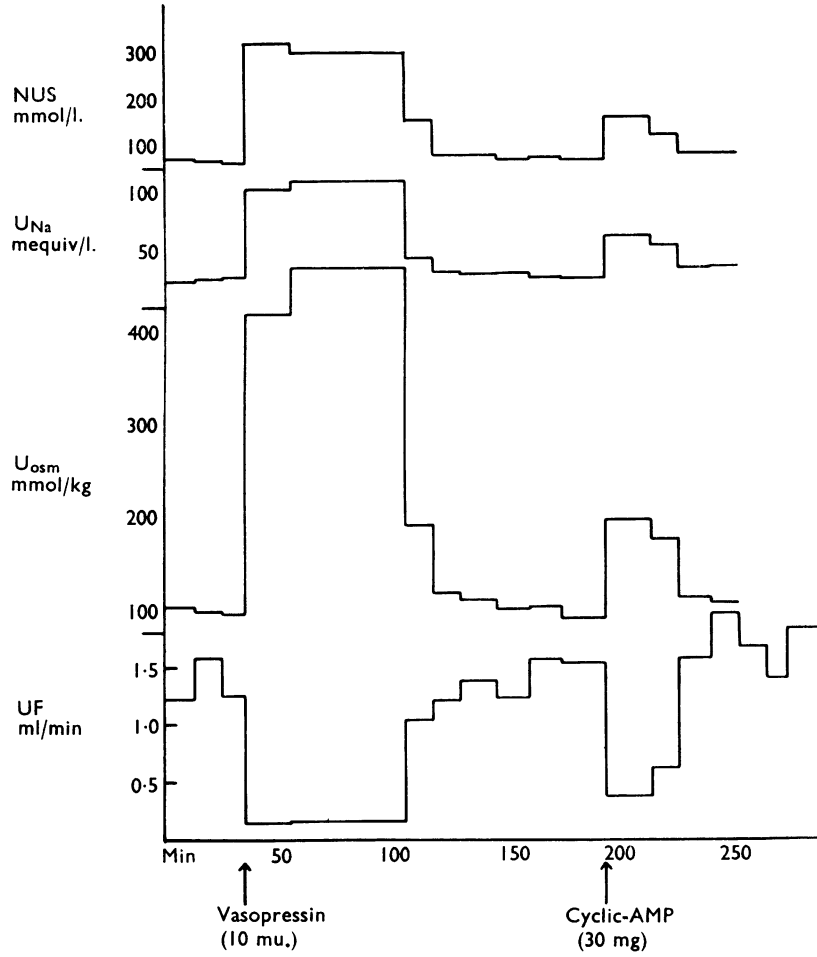

FIG. 1. Effects of vasopressin (10 mu.) and cyclic-AMP (30 mg) on urine flow (UF), urine osmolality (U_{osm}), and on the urinary concentrations of sodium (U_{Na}) and non-urea solute (NUS). Rabbit C2.

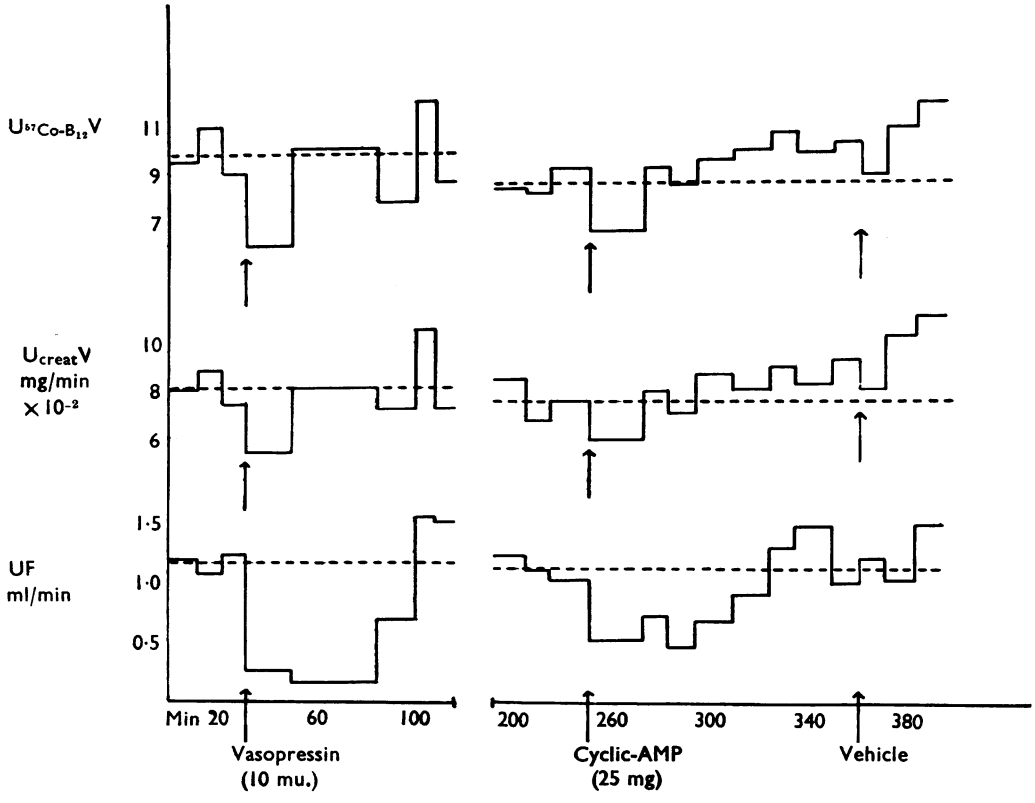

FIG. 2. Similar effects of vasopressin and cyclic-AMP on urine flow (UF), and on the excretion rates of the "glomerular substances" creatinine ($U_{creat.}V$) and ^{57}Co-cyanocobalamin $U^{57}Co\text{-}B_{12}$ (V). 1 mg of unlabelled cyanocobalamin was given intravenously 1 h before starting infusion of the isotope. The "vehicle" was the solvent used to administer cyclic-AMP. Each broken line represents the mean of three control periods. Rabbit B.

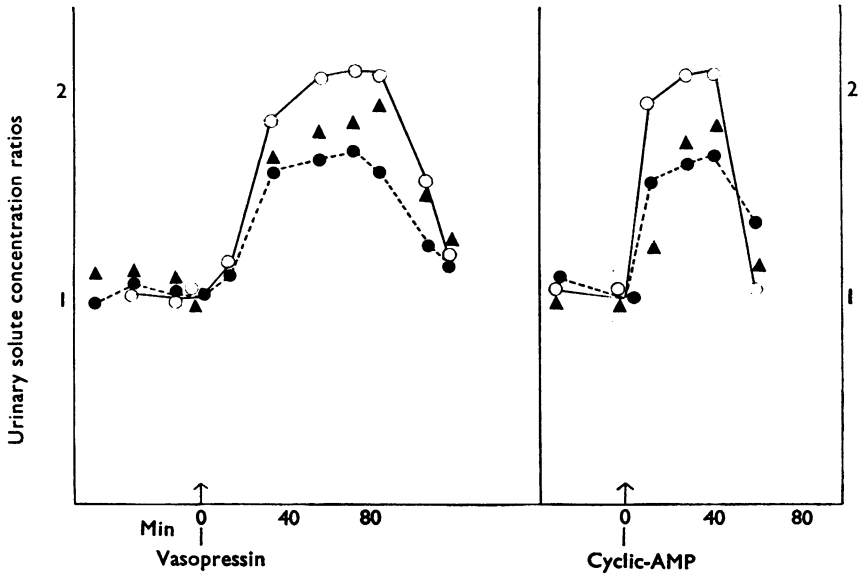

FIG. 3. Effects in rabbit F of vasopressin (5 mu.) and cyclic-AMP (30 mg) on urinary solute concentration ratios for creatinine (○——○), urea (●– – –●), and osmolality (▲).

by vasopressin. It is unlikely that the changes observed with cyclic-AMP in our studies were due to the endogenous release of vasopressin. There is no *a priori* reason to suspect a physiological role for cyclic-AMP in releasing vasopressin and the animals appeared undisturbed by the injections. Moreover Levine (1967) observed that cyclic-AMP reduced urine flow in two patients with diabetes insipidus.

One of the characteristic effects of vasopressin is to increase the fraction of filtered urea reabsorbed by the renal tubules. This can be shown during an acute vasopressin-mediated antidiuresis by comparing the changes in urinary concentration of urea with those of substances such as inulin or creatinine which are not reabsorbed by the renal tubules (Thomas, 1964 ; Jones, Barraclough, Perriello & Marsden, 1967). As urine flow falls due to the reabsorption of water, the concentration of urea in the urine rises less than that of creatinine. The same phenomenon was seen after administration of cyclic-AMP, which also increased the fraction of filtered urea reabsorbed by the renal tubules.

Although the similarity of the changes in urine flow and composition produced by vasopressin and cyclic-AMP are consonant with a similar mode of action on the kidney, the possibility that the antidiuresis of cyclic-AMP was due to a fall in glomerular filtration rate must also be considered. The abrupt reduction in urine flow produced by both vasopressin and cyclic-AMP precluded the valid measurement of renal clearances due to errors in dead space and flow. However, cyclic-AMP and vasopressin had similar effects on creatinine excretion in every experiment, and on the excretion of ^{57}Co-cyanocobalamin (Nelp, Wagner & Reba, 1964) in the one rabbit also studied with this " glomerular substance " (Fig. 2). As vasopressin is believed not to affect the glomerular filtration rate, these results

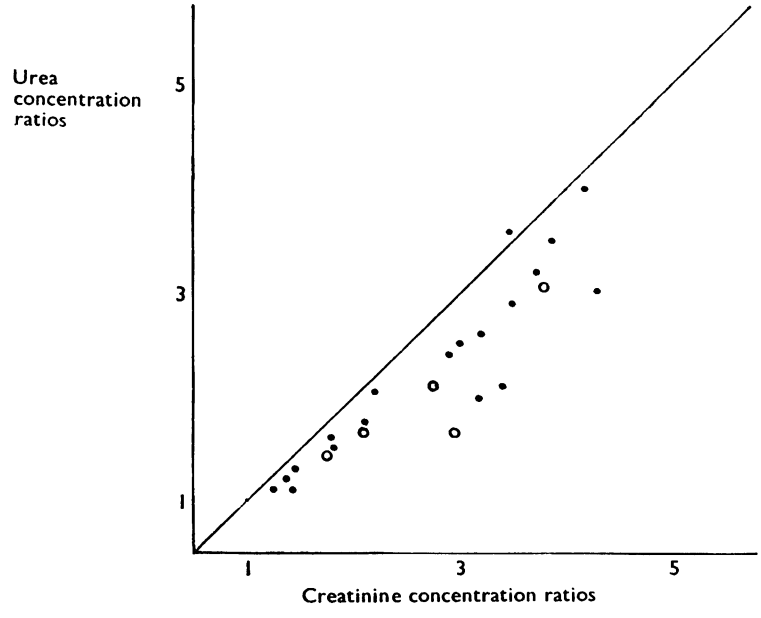

FIG. 4. Urinary concentration ratios for creatinine and urea are plotted during antidiuresis produced by vasopressin (●) in nine rabbits, and by cyclic-AMP (○) in five rabbits. The diagonal line represents a 1 to 1 relationship between these two variables. Cyclic-AMP is shown to have a quantitatively similar effect to vasopressin in lowering the urea/creatinine concentration ratio.

suggest that cyclic-AMP also had little effect on this function. Moreover a fall in glomerular filtration rate usually causes a fall in urinary sodium concentration (Mueller, Surtshin, Carlin & White, 1951), whereas the latter increased in response to cyclic-AMP in four of the five experiments. The nature of the antidiuresis following intravenous infusion of cyclic-AMP is consistent with the view that vasopressin mediates its action via cyclic-AMP.

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