Plasma adenosine 3′,5′-cyclic monophosphate in human hypertension

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Summary: In a previous study we observed an increase in urinary cyclic AMP in labile hypertension in the upright position and during isoproterenol infusion, in contrast to a decrease in control subjects. In the present study we measured the plasma level of cyclic AMP in control subjects and patients with various types of hypertension. We obtained the following results: (1) plasma cyclic AMP increases in response to upright posture in control subjects and hypertensive patients; (2) values of cyclic AMP in the recumbent and upright positions are comparable in control subjects and patients with essential hypertension, but are significantly higher in those with true renovascular hypertension due to bilateral renal artery stenosis; (3) propranolol inhibits the increase of plasma cyclic AMP in response to posture in control subjects, but has an opposite effect in labile hypertension where there is a further increase; (4) the rise in blood pressure in pheochromocytoma is associated with a considerable increase in plasma cyclic AMP.

Present and previous data suggest that kidney handling of cyclic AMP is abnormal in hypertension, and that the specific defect may be related to the type of hypertension.

Résumé: Nous avons précédemment décrit que l'excrétion de l'AMP cyclique urinaire augmente en position debout et lors de l'infusion à l'isoprotérénol dans l'hypertension labile. Une réponse opposée est survenue chez les sujets de contrôle. Dans la présente étude, nous avons entrepris les mesures de l'AMP cyclique dans le plasma chez des sujets souffrant de différents types d'hypertension. Nous avons observé les faits suivants: (1) l'AMP cyclique plasmatique augmente en position debout tant chez les sujets de contrôle que chez les hypertendus; (2) les niveaux de l'AMP cyclique plasmatique dans les positions couchée et debout chez les sujets de contrôle sont comparables à ceux obtenus chez les patients avec hypertension essentielle, tandis que des taux significativement élevés ont été trouvés chez ceux avec hypertension rénovasculaire (avec sténose bilatérale de l'artère rénale); (3) le propranolol empêche l'augmentation posturale de l'AMP cyclique chez les normaux, tandis qu'il accentue la réponse posturale dans l'hypertension labile; (4) le paroxysme hypertensif dans un cas de phéochromocytome a été accompagné d'une augmentation considérable de l'AMP cyclique plasmatique.

Les résultats présents et antérieurs suggèrent que le rein dispose de façon anormale de l'AMP cyclique dans l'hypertension et ceci possiblement de façon différente selon le type d'hypertension.

The secretion and/or cellular action of several blood-pressure regulating substances (renin, angiotensin, aldosterone, catecholamines, prostaglandins and others) are mediated by adenosine 3′,5′-cyclic monophosphate (cyclic AMP).[1] In previous studies we reported an increase of urinary cyclic AMP excretion in response to upright posture and isoproterenol in labile hypertension,[2] and to upright posture in stable and renovascular hypertension;[3] no such change occurred in control subjects who showed, on the contrary, a small decrease in urinary cyclic AMP excretion. Propranolol reversed the abnormal response in patients with labile hypertension to the pattern observed in control subjects.[4]

In order to elucidate the differences observed we measured plasma cyclic AMP in control subjects and in different groups of hypertensive patients during recumbency and after assumption of upright posture.

Methods

Subjects

The group of controls comprised six volunteers who were young healthy subjects with no history of hypertension in parents or relatives. A complete physical examination revealed no abnormalities and blood pressure was below 135/90 mm Hg. Basic laboratory data, including hemogram and urinalysis results, were within normal limits.

Thirteen patients with benign essential hypertension

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(Stages I and II, WHO classification) were completely investigated before entering the study to eliminate any cases of secondary hypertension. None had retinopathy, and all had normal serum Na^+, K^+ and total CO_2^{2-}. Left ventricular hypertrophy was the only acceptable anomaly in the ECG. Renal arteriography and rapid sequence intravenous pyelography yielded normal findings, and renal function was normal or only slightly decreased. There were no overt signs of arterioatherosclerosis of large vessels. This group was further subdivided into labile and stable hypertensives based on the response of blood pressure to hospitalization and rest. Six patients with levels decreasing below 140/90 mm Hg during hospitalization and recumbent posture were considered to be "labile", while seven patients with blood pressure remaining over this limit under the same conditions were considered to be "stable".

Four patients with bilateral renal artery stenosis, with at least 60% stenosis on one side, presented a more severe degree of hypertension (Table I). The renovascular origin of hypertension was confirmed in two patients by a considerable decrease in blood pressure to almost normal levels in response to surgical treatment. In one patient who had not been surgically treated the plasma renin activity (PRA) in the venous blood from the more affected kidney (60% stenosis) was almost double that from the less affected one (30% stenosis). The fourth patient had a considerable increase in the peripheral plasma angiotensin II concentration. Endogenous creatinine clearance was normal in all four patients, being 94, 93.2, 98 and 94.6 ml/min respectively.

One patient with paroxysmal hypertension due to a surgically confirmed adrenal pheochromocytoma was also included in our study. In addition to exploring repeatedly the effect of upright posture under conditions of "normotension", we also studied this patient during a hypertensive crisis associated with a greatly increased plasma norepinephrine level.

Protocol of investigation

Conditions: All subjects received during the period of the study a standard diet containing 135 mEq of sodium and 90 mEq of potassium per day. Smoking and beverages containing xanthines were forbidden for at least 12 hours prior to plasma sampling.

Effect of body position: This study was performed on the fourth and fifth days while the subjects were on controlled diets. The subjects had been recumbent since 23:00 the preceding evening. Plasma samples were drawn one day at 12:30 after four hours of recumbency, and the other day with the subject up and walking leisurely during the entire four-hour period with only short intervals of sitting. The sequence of positions was randomized. Blood was drawn into prechilled tubes containing 15 mg of ethylenediaminetetra-acetic acid in order to inhibit destruction of cyclic AMP and immediately centrifuged at 4°C. Plasma was separated and frozen at −70°C for later assay of cyclic AMP. In the patient with pheochromocytoma, urine samples were collected on ice between 8:30 and 12:30 while the patient was in the recumbent and upright positions, and immediately frozen at −70°C.

Effect of propranolol: This study was performed in three control subjects and six patients with labile hypertension. The same conditions of bed rest and diet were applied, but the protocol was continued for 10 days and the effect of body position was studied on the fourth, fifth, ninth and tenth days. The study was divided into two periods of five days during which the subjects received either propranolol 40 mg four times a day or an identical placebo* in alternate periods. The sequence of position and of administration of medication or placebo were randomized and the study conducted in a double-blind fashion.

Cyclic AMP measurements

Plasma and urinary cyclic AMP were measured by the protein-binding method of Gilman.[5] Urine was diluted 5 to 10 times with 0.05 M sodium acetate/acetic acid buffer, pH 4, as previously described.[2] Plasma was quickly thawed, and to 4 ml was added 0.01 μCi of ^{3}H-cyclic AMP (specific activity 22.1 Ci/mM of cyclic AMP). Proteins were then precipitated by adding 25 ml of cold tridistilled ethanol 99%. After centrifugation for 15 minutes (15,000 G at 4°C) the supernatant was evaporated under a vacuum at 45°C. The residue was then suspended in 2 ml of sodium acetate/acetic acid buffer (50 mM, pH 4.0), centrifuged for 10 minutes at 15,000 G, and the supernatant lyophilized. The dry residue was suspended in 2 ml of distilled water and centrifuged; the supernatant (100 μl) was used for recovery studies and incubated in

*Propranolol and placebo tablets were kindly supplied by M. R. Dufresne, M.D., Ayerst Laboratories, Montreal

Table I—Data of four patients with hypertension and bilateral renal artery stenosis

Patient	Age	Sex	Degree of renal artery stenosis		Blood pressure*		
			Left	Right			
L.R.	50	F	20%	main artery 60%, accessory artery 70%	206/122 155/101	(preoperative) (postoperative)	
B.C.	43	M	70%	85% (with poststenotic dilatation)	185/116 158/93	(preoperative) (postoperative)	
H.L.	58	M	60% (with poststenotic dilatation)	30%	185/105	PRA (ng/ml):	left renal vein 100 right renal vein 60 inferior vena cava 50
C.B.	59	F	50%	80% (with poststenotic dilatation)	195/115	Plasma angiotensin II in peripheral blood 740 pg/ml†	

*Mean of at least 10 blood pressure readings in recumbent position.
†Plasma angiotensin II (radioimmunoassay) values in control subjects under the same conditions (135 mEq Na and 90 mEq K/day in diet; recumbency) are between 10 and 16 pg/ml.
PRA = plasma renin activity (after 18 hours of incubation).

triplicate with different quantities of unknown (e.g. 100 μl, 150 μl, 100 μl + 10 pM of cyclic AMP). The rest of the procedure was identical to that described by Gilman.[5]

The specificity of the assay was verified by remeasurement of cyclic AMP content of representative samples after incubation for 12 hours with 3′,5′-cyclic nucleotide phosphodiesterase (Sigma), 0.25 U of enzyme/ml of plasma, at 30°C, pH 7.5, and with 20 mM $MgSO_4$. Using the previously described purification procedure phosphodiesterase-treated samples were found to contain no detectable cyclic AMP.

The intra-assay coefficient of variation between the three different dilutions was 6.84% for urinary values and 16.61% for plasma values; a mean of the three dilutions is presented. The coefficient of variation of "pool plasma" (measured with each run) was 3.89 ± 0.12%. The mean recovery of cyclic AMP added to a sample of plasma was 79.5% (standard error 2.1, n = 75). Every sample was corrected for its recovery. The inter-assay coefficient of correlation was significant with the enzymatic assay,[6] $r = 0.59$, $P < 0.05$, and with the radioimmunoassay,[7] $r = 0.98$, $P < 0.001$. Statistical analysis was performed using unpaired "t" tests, (a) comparing control subjects and patients with renovascular hypertension, in both the recumbent and upright positions, and (b) comparing patients with essential hypertension and renovascular hypertension, in both positions. A paired "t" test was performed to analyse the effect of position on plasma concentration of cyclic AMP in all subjects.

Results

Plasma cyclic AMP levels during recumbency and in response to upright posture

Table II sets forth the pertinent data in control subjects and patients with renovascular hypertension, including plasma cyclic AMP concentration during recumbency and in response to upright posture. Table III sets forth the same data in patients with essential hypertension, grouped into those with labile and stable forms.

In all subjects upright position had no significant effect on pulse rate or mean blood pressure, but caused an increase (mean 28.6 ± 4.9%) in plasma cyclic AMP, significant at $P < 0.01$. (This increase, however, is not significant if hypertensive subjects are considered separately.) While there were no significant differences between control subjects and those with benign essential hypertension in both recumbent and upright positions, there were, as illustrated in Fig. 1, significantly higher plasma cyclic AMP values in subjects with renovascular hypertension in recumbent ($P < 0.001$ comparing control subjects and $P < 0.001$ comparing essential hypertension) and upright ($P < 0.02$ comparing control subjects and $P < 0.001$ comparing essential hypertension) positions. This increased plasma level was present in spite of a grossly normal total glomerular filtration as estimated by the endogenous creatinine clearance (94.95 ± SEM 1.05 ml/min).

If we consider the urinary excretion of cyclic AMP as well as plasma values, we can grossly estimate the clearance of endogenous cyclic AMP (Table IV). It was lower in those with renovascular hypertension than in controls, as was the ratio of clearances of cyclic AMP and creatinine (1.13 ± 0.21 *v.* 1.68 ± 0.19). However, because of the small sample size and variation, those results are not statistically significant.

The effect of propranolol on plasma cyclic AMP and its response to upright posture in control subjects and labile hypertensives

Mean results from control subjects and patients with labile essential hypertension are shown in Fig. 2. Control subjects presented under placebo administration an increase in plasma cyclic AMP comparable to that previously described. Propranolol administration not only prevented

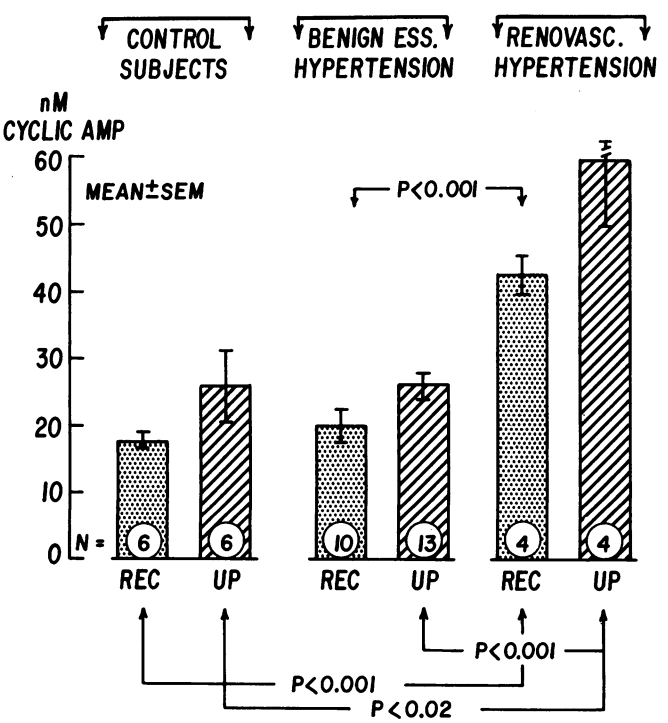

FIG. 1—Plasma cyclic AMP in nanomoles per litre (nM) in control subjects and patients with essential or renovascular hypertension. N = number of patients in every group.

Table II—Data of control subjects and patients with renovascular hypertension

Name	Age	Sex	MBP	PR	cAMP*	MBP	PR	cAMP
			Recumbent			Upright		
Control subjects								
T.N.	24	F	72	74	17.56	78	85	22.56
D.J.	22	F	69	82	15.62	76	74	16.03
D.O.	22	F	72	76	23.33	76	88	55.00
N.Y.	21	M	97	91	17.10	85	100	22.26
L.L.	23	F	68	68	20.26	70	74	23.21
E.M.	21	M	77	82	17.46	90	58	19.56
Mean			75.8	78.8	18.55	79.2	79.8	26.43
± SEM			4.4	3.2	1.13	2.9	5.9	5.81
Patients with true renovascular hypertension								
H.L.	58	M	102	76	47.74	104	84	91.24
C.B.	59	F	138	69	43.39	112	73	55.29
L.R.	50	F	136	74	35.76	140	96	48.69
B.G.	43	M	156	68	48.52	126	80	47.32
Mean			133.0	71.7	43.85	120.5	83.7	60.63
± SEM			11.8	1.9	2.92	7.9	4.8	10.34

*Plasma cyclic AMP in nanomoles per litre.
MBP = mean blood pressure in mm Hg (diastolic + ⅓ of pulse pressure).
PR = mean pulse rate per minute during the same period.

this increase but also resulted in a decrease in the response to upright position.

Labile hypertensive patients presented under placebo administration a slight but not significant increase in plasma cyclic AMP in response to upright posture, similar to that seen in benign essential hypertension. With propranolol administration, however, the plasma cyclic AMP increase was not abolished, as in control subjects, but, on the contrary, was even greater in response to upright posture.

Plasma and urinary cyclic AMP levels in pheochromocytoma

As seen from Table V, the level of plasma cyclic AMP during recumbency and in response to upright posture was comparable to that of control subjects and patients with benign essential hypertension. In response to upright posture the urinary cyclic AMP remained virtually unchanged. A spontaneous paroxysmal hypertensive crisis was associated with a seven- to tenfold increase in the level of plasma cyclic AMP.

Discussion

The system of the "second messenger" has only occasionally been investigated in hypertension, and the urinary excretion of cyclic AMP was reported by Taylor *et al*[8] to be normal in hypertension; we have confirmed this. However, when the effects of upright posture and isoproterenol administration were studied, urinary cyclic AMP excretion was found to increase in patients with labile hypertension and to decrease in control subjects.[2]

Our findings indicate that all control subjects and hypertensive patients responded to upright posture by an increase in the level of plasma cyclic AMP of the order of (mean) 28.6 ± 4.9%. This increase is large enough to be detectable with precision by the assay used (coefficient of variation 3.89 ± 0.12%).

It is difficult to explain the qualitative differences between these groups in the urinary excretion in response to upright posture by any mechanism other than a change in the renal secretion or handling of cyclic AMP. The upright position is associated with, among other physiologic changes, adrenergic stimulation.[9] Beta-adrenergic stimulation was shown in normal subjects to induce an increase in plasma cyclic AMP without a corresponding increase in urinary cyclic AMP;[10] this would imply increased tubular reabsorption of cyclic AMP or an important decrease of "nephrogenous" cyclic AMP. About 80% of urinary cyclic AMP is derived, under control conditions, from the glomerular filtrate, the remainder being of tubular origin. We even observed after prolonged stimulation with isoproterenol a decrease in the urinary cyclic AMP excretion in control subjects.[3]

Our measurements of plasma cyclic AMP in control subjects indicate that postural stimulation results in increased tubular reabsorption or decreased tubular excretion of urinary cyclic AMP. The plasma cyclic AMP responses to upright posture in essential hypertension (including labile hypertension) are comparable to those in control subjects. This would mean that the difference in

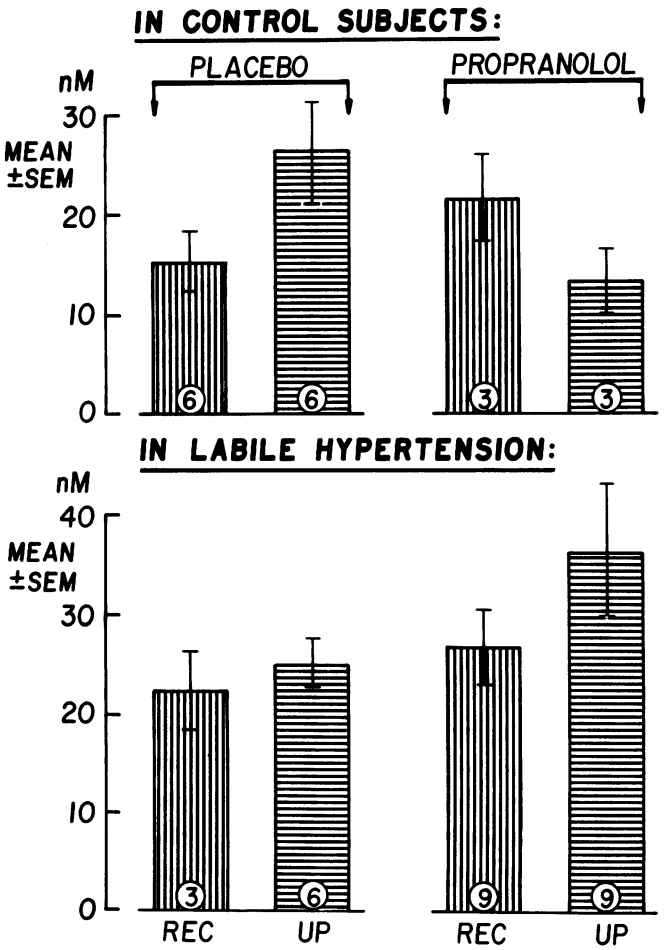

FIG. 2—Plasma cyclic AMP in nanomoles per litre (nM) in control subjects and labile hypertensive patients under placebo and propranolol treatment, recumbent (REC) and upright (UP). The numbers of patients in every group are in circles.

Table III—Data of patients with essential hypertension

Name	Age	Sex	MBP	PR	cAMP	MBP	PR	cAMP
			Recumbent			Upright		
Labile								
P.G.	30	M	104	69	—	112	96	28.5
V.D.	49	F	105	95	20.81	97	115	21.36
H.A.	30	M	100	67	15.48	101	76	17.75
C.A.	21	M	96	81	30.41	104	93	35.50
G.J.	12	F	81	82	—	84	96	25.76
F.N.	15	M	96	81	—	73	93	20.60
R.A.	47	F	109	102	20.28	112	91	24.70
Mean			98.7	82.4	21.74	97.6	94.3	24.88
± SEM			3.5	4.8	3.12	5.5	4.3	2.22
Stable								
R.D.	22	F	117	87	17.30	118	99	26.87
D.J.L.	52	M	132	86	19.72	122	93	29.74
L.F.	41	M	111	80	36.73	108	86	47.46
H.G.	50	F	106	77	9.36	107	82	16.02
L.M.	24	M	105	92	22.12	111	101	28.60
D.M.	18	M	143	78	11.66	129	108	17.34
Mean			119	83.3	20.39	115.8	94.8	26.16
± SEM			6.3	2.4	2.58	3.5	4.0	2.36
All patients								
Mean			108.1	82.8	20.38	106	94.5	26.16
± SEM			4.4	2.7	2.58	4.2	2.8	2.37

the response of urinary cyclic AMP to upright posture is not due to a change in plasma cyclic AMP but to a change in tubular reabsorption or secretion of cyclic AMP in these patients. This interpretation is limited by the fact that we are not presenting urinary and plasma results together. Unfortunately, in the majority of our patients with essential hypertension the cyclic AMP excretion was measured by radioimmunoassay[2] and plasma levels by protein-binding assay. In spite of a good qualitative correlation between the two methods of assay, quantitative comparison may be inaccurate. Further study, including direct measurement of clearance of cyclic AMP, is therefore needed.

In renovascular hypertension we have previously observed a normal urinary cyclic AMP excretion in both recumbent and upright positions.[3] The increase we have observed in plasma cyclic AMP in both positions may indicate an increased cellular cyclic AMP production or a decreased destruction and/or excretion of this nucleotide in this type of hypertension. The first possibility would be compatible with some evidence that cyclic AMP is an intracellular mediator of the action of catecholamines on renin release,[11] with the role of the renin-angiotensin system in this type of hypertension, and with the fact that cyclic AMP has an effect on sodium transport similar to aldosterone,[12] another hormone known to be elevated in renovascular hypertension. The abnormal handling of cyclic AMP is further suggested by a decrease in clearance of endogenous cyclic AMP (Table IV). Since the kidney was recently identified[22,23] as a site of cyclic AMP elimination as well as production, it is conceivable that disturbances may occur with significant renal artery stenosis.

Propranolol is a useful therapeutic tool, particularly in the treatment of labile hypertension.[13] We have shown that propranolol can, in addition, normalize two clinical (blood pressure and pulse rate) as well as two humoral parameters (PRA and urinary cyclic AMP response to upright posture).[4] In the present study propranolol treatment was shown to abolish the plasma cyclic AMP increase in response to upright posture in control subjects, but no such effect could be observed in labile hypertensive patients; on the contrary, the plasma cyclic AMP increased in response to upright posture during propranolol administration. To reconcile these observations with previous findings in the urine[4] one must conclude that propranolol did not "normalize" cyclic AMP responses, at least not at the plasma cyclic AMP level. If a reversal to "normal" patterns in urinary cyclic AMP excretion occurred under propranolol, such a change in tubular secretion and/or reabsorption probably had a secondary effect on the plasma cyclic AMP response to upright posture, which is qualitatively different from the response in control subjects. Only further studies of the metabolism of cyclic AMP and of the control of its excretion will show whether a primary abnormality in the "second messenger" system exists in patients with hypertension. Patients with labile hypertension and excessive adrenergic responsiveness seem to be, as previously shown,[14] possible examples of such an abnormality.

Another type of hypertension in which cyclic AMP abnormality is certainly secondary to catecholamine excess is pheochromocytoma. When the patient is not experiencing attacks of hypertension, urinary and plasma levels of cyclic AMP in both positions are comparable to those in control subjects and in patients with essential hypertension. During the attack, the rapid release of catecholamines (in this case not only norepinephrine but also a considerable amount of epinephrine, a typical β-adrenergic catecholamine) leads to a very significant increase in plasma cyclic AMP. The observed patterns of plasma cyclic AMP levels may not necessarily be present in patients with tumours producing predominantly α-adrenergic catecholamines.

Our work demonstrates the importance of concomitant studies of plasma and urinary cyclic AMP under dynamic conditions. Previous studies of plasma and urinary catecholamines in hypertension failed in most cases to demonstrate a relationship between the degree of hypertension and catecholamine excess.[15] It was shown, however, that the sympathetic-parasympathetic interaction, which may be important in the pathogenesis of hypertension,[16] may be regulated by cyclic nucleotides.[17-19] Cyclic AMP is a vasodilating nucleotide and its role in hypertension may be different in the arterioles (where a decrease of cyclic AMP is associated with α-stimulation and vasoconstriction) and in the heart (where its increase is associated with β-adrenergic stimulation and increased heart rate, contractility and cardiac output). Therefore, these two different tissue responses, possibly cyclic-AMP-mediated, have to be considered in order to understand and reconcile findings such as a decrease in arterial cyclic AMP in spontaneously hypertensive rats,[20] with present data pointing rather to increased plasma and urinary cyclic

Table IV—Urine and plasma levels and clearance of cyclic AMP

				Clearance ¶	
Name	PcAMP*	UcAMP†	Volume‡	cAMP	creatinine
Control subjects					
T.N.	17.56	3.32	3.60	184.7	102.5
D.J.	15.62	3.92	3.60	207.4	95
N.Y.	17.10	2.67	1.41	156.4	109
E.M.	17.46	1.96	1.50	112.4	85
Mean	16.93	2.96	2.52	165.21	97.87
± SEM	0.45	0.42	0.62	20.5	5.2
True renovascular hypertension					
H.L.	47.74	2.32	4.04	48.6	94.0
C.B.	43.39	5.22	4.1	120.4	93.2
L.R.	35.76	4.92	3.2	137.7	98.0
B.G.	48.52	6.04	3.8	124.7	94.6
Mean	43.85	4.62	3.78	107.85	94.95
± SEM	2.92	0.8	0.20	20.08	1.05

*Plasma cyclic AMP in nanomoles per litre.
†Urinary cyclic AMP in nanomoles per minute.
‡Urinary volume in ml/min.
¶Clearance of substance in ml/min.

Table V—Urine and plasma cyclic AMP levels in a 34-year-old man (B.J.) with pheochromocytoma of the left adrenal gland

Period of "normotension"	Urinary cAMP nM/min	Plasma cAMP nM/l	BP mm Hg
Recumbent	7.88	20.4	160/90
Upright	6.17	29.3	150/95
Recumbent	4.40	21.3	155/80
Upright	4.23	25.4	150/90
During a hypertensive crisis* (recumbent)	—	135.6	240/130
	—	266.9	230/135

*Associated with an increase in plasma norepinephrine up to 2.6 ng/ml (upper limit of normal in control subjects is 0.43 ng/ml) and an increase in urinary epinephrine up to 342 μg/24 hr (normal up to 25 μg/24 hr).

AMP in some types of hypertension. In addition, it must be stressed that essential hypertension is a heterogeneous entity and cyclic AMP is not the only cyclic nucleotide involved in the "second messenger" system; other nucleotides, like cyclic guanosine monophosphate,[21] have to be considered as well. Further concurrent studies of cyclic AMP and cyclic GMP and of the enzymes controlling their levels may offer a better insight into the balance of adrenergic and parasympathetic activities important in the regulation of blood pressure.

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