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Abstract

Urinary excretion of cyclic GMP (cGMP) and the plasma level of cyclic AMP (cAMP) were determined in patients with liver diseases. The urinary excretion of cGMP, expressed on the basis of creatinine excreted per day, was at significantly higher levels not only in primary hepatoma but also in liver cirrhosis, while the plasma level of cAMP was higher only in liver cirrhosis. Thus, the ratio of urinary cGMP excretion to plasma cAMP level in primary hepatoma was significantly higher than that in liver cirrhosis. In cirrhotic patients studied by catheterization, the level of cGMP in the hepatic vein was significantly lower than that in the superior mesenteric or portal vein, indicating the uptake of cGMP by the liver. Since cGMP excretion correlated with KICG both in liver cirrhosis and primary hepatoma, the increased cGMP excretion appeared to be explained by a reduced uptake of cGMP by the liver.

KEYWORDS: urinary cGMP, plasma cAMP, hepatitis, liver cirrhosis, hepatoma

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INCREASED EXCRETION OF URINARY CYCLIC GMP IN PRIMARY HEPATOMA AND PRENEOPLASTIC LIVER†

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Abstract. Urinary excretion of cyclic GMP (cGMP) and the plasma level of cyclic AMP (cAMP) were determined in patients with liver diseases. The urinary excretion of cGMP, expressed on the basis of creatinine excreted per day, was at significantly higher levels not only in primary hepatoma but also in liver cirrhosis, while the plasma level of cAMP was higher only in liver cirrhosis. Thus, the ratio of urinary cGMP excretion to plasma cAMP level in primary hepatoma was significantly higher than that in liver cirrhosis. In cirrhotic patients studied by catheterization, the level of cGMP in the hepatic vein was significantly lower than that in the superior mesenteric or portal vein, indicating the uptake of cGMP by the liver. Since cGMP excretion correlated with K_{ICG} both in liver cirrhosis and primary hepatoma, the increased cGMP excretion appeared to be explained by a reduced uptake of cGMP by the liver.

Key words : urinary cGMP, plasma cAMP, hepatitis, liver cirrhosis, hepatoma.

Both cyclic adenosine 3', 5'-monophosphate (cAMP) and cyclic guanosine 3', 5'-monophosphate (cGMP) have been implicated in regulation of cell growth and differentiation. Generally, cAMP acts as a negative signal and cGMP as a positive one for cell proliferation. Consistent with this is the finding that some Morris hepatomas have higher tissue levels of cGMP and/or cGMP/cAMP ratios compared with normal liver tissue (1-3). However, a positive role for cAMP in ethionine-induced hepatoma and preneoplastic liver has also been proposed (4-6).

Murad *et al.* (7) and Criss *et al.* (8) reported increased excretion of urinary cGMP, but not of cAMP, in rats bearing hepatomas and showed that a significant correlation existed between the cGMP excretion and tumor size. Neethling *et al.* (9) made a similar observation on three patients with primary hepatoma.

Since primary hepatoma in Japan is characterized by having post-hepatitis cirrhosis of the liver in more than 80 % of such cases (10), cirrhosis can be

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regarded as a preneoplastic state. This also has been suggested by histological (11) and enzymological studies of our own (12, 13).

It is, therefore, of some interest to study the urinary excretion of cGMP in cases with liver cirrhosis as well as primary hepatoma. The present communication reports increased excretion of cGMP in both hepatoma and liver cirrhosis. Plasma cAMP level was elevated only in the latter. The mechanisms of the increased urinary excretion of cGMP in liver cirrhosis was explored by analyzing the levels of cyclic nucleotides in portal and hepatic veins. The results have been reported previously in abstract form (14).

MATERIALS AND METHODS

Of the subjects studied, 22 had acute hepatitis (12 females, 10 males, 14-69 years old), including 19 cases of acute stage with serum transaminase levels higher than 300 IU or serum bilirubin levels higher than 5 mg/100 ml and 3 cases of recovery stage, 15 had chronic hepatitis (5 females, 10 males, 22-63 years old), including 1 alcoholic, 2 with chronic persistent and 12 with chronic active hepatitis, 35 had liver cirrhosis (5 females, 30 males, 8-68 years old), including 3 with Wilson's disease and 32 with post-hepatitis liver cirrhosis cases, 26 had primary hepatoma with liver cirrhosis (2 females, 24 males, 35-82 years old) and 13 were normal controls (5 females, 8 males, 23-59 years old). The diagnoses were established by histological examination for all cases of chronic hepatitis, 25 of those with liver cirrhosis, 13 of those with acute hepatitis and 9 of those with primary hepatoma (hepatocellular carcinoma) on tissues obtained by biopsy, surgical resection or autopsy. The rest were diagnosed by routine laboratory tests and other available radiological and physical techniques.

Twenty-four hour urine specimens for cGMP assay were collected at room temperature in bottles containing 10 ml of 0.5 M ethylenediamine tetraacetic acid (EDTA), pH 7.4. Blood samples for cAMP assay were taken in the morning, when the 24 h urine collection had been completed, in test-tubes receiving an EDTA solution to give a final concentration of 5-10 mM of EDTA. Plasma was separated immediately by centrifugation at 4℃. These specimens were stored at −20℃ until analyzed.

cAMP and cGMP concentrations in plasma and cGMP concentration in urine were determined by radioimmunoassay using Yamasa Cyclic AMP Assay Kit and Yamasa Cyclic GMP Assay Kit (Yamasa Syoyu Co., Ltd. Chiba), respectively (15). Urine concentration of creatinine was determined on the same specimens by the Jaffe reaction (16). Plasma cAMP and cGMP concentrations were expressed in nmoles/100 ml plasma and pmoles/100 ml plasma, respectively. Urinary cGMP excretion was expressed in μmoles/g creatinine/day.

Glucagon levels in over-night fasting plasma were determined by radioimmunoassay with antibody against 30 K glucagon at Otsuka Assay Laboratory (Otsuka Pharmaceutical Co., Ltd., Tokushima) on 6 cases of liver cirrhosis and 9 cases of acute hepatitis among those studied for plasma cAMP level.

The blood samples were taken from splenic, superior mesenteric, portal and peripheral veins in percutaneous transhepatic portography (PTP) from 10 patients with liver cirrhosis (3 females, 7 males, 36-70 years old) and from a patient with a nonspecific liver disorder with a past history of splenomegaly (male, 48 years old). These samples were also analyzed for plasma cAMP and cGMP. In 2 of them, hepatic venous blood was obtained simultaneously in PTP and in 2 other cases hepatic venous and peripheral arterial blood samples

were also obtained by hepatic vein catheterization.

The cyclic nucleotide values in the plasma and urine in hepatoma patients spread over a wide range, especially the ratio of urinary cGMP to plasma cAMP. A nonparametric, distribution free approach was therefore selected to determine the significance levels of differences in cyclic nucleotide levels and ratios between the two liver diseases (17). The results are given as the mean ± standard error of the mean (SEM).

RESULTS

Plasma cAMP levels in controls and liver disease patients are shown in Fig. 1. A significantly higher value was found only in liver cirrhosis. A considerably wide distribution of individual values found among each group could not be attributed to the difference in disease activity, since no significant correlation was found between the cAMP level and the results of liver function tests.

In contrast to the change in plasma cAMP concentration, the urinary excretion of cGMP was significantly higher in both liver cirrhosis and primary

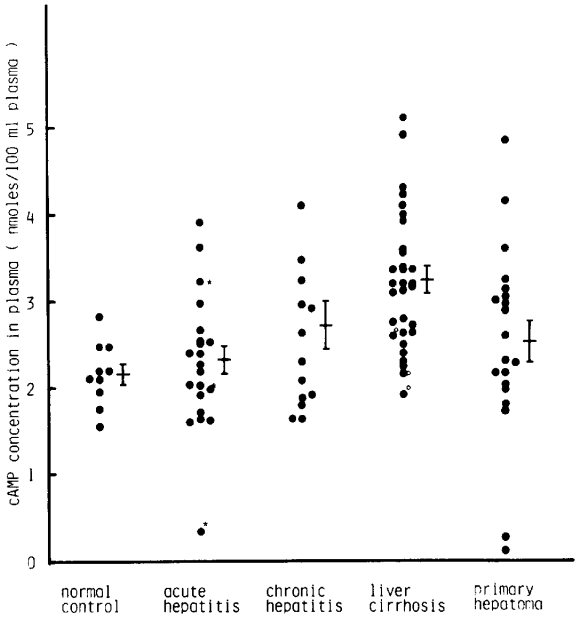

Fig. 1. Plasma cAMP levels in patients with liver diseases on admission or on first determination. Mean and SEM in nmoles/100 ml plasma are: normal controls, 2.17 ± 0.12; acute hepatitis, 2.30 ± 0.17; chronic hepatitis, 2.51 ± 0.22; liver cirrhosis, 3.22 ± 0.14; primary hepatoma, 2.51 ± 0.25. cAMP level markedly increased in the terminal stage of primary hepatoma and such values taken within 1 week before death are not included. Significance levels of differences in cAMP concentration between liver cirrhosis and other groups were: $p < 0.001$ for normal controls and acute hepatitis, $p < 0.01$ for chronic hepatitis and $p < 0.05$ for primary hepatoma. The cAMP levels of the recovery cases of acute hepatitis* and those of Wilson's disease° were not different from those of the rest of the cases in respective groups.

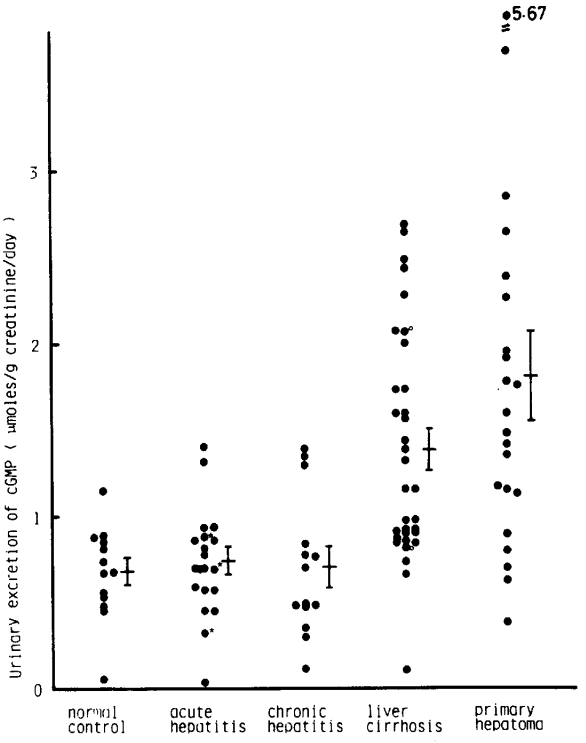

Fig. 2. Urinary excretion of cGMP in patients with liver diseases on admission or on first determination. Mean and SEM in μmoles/g creatinine/day are: normal controls, 0.67 ± 0.07; acute hepatitis, 0.73 ± 0.07; chronic hepatitis, 0.70 ± 0.11; liver cirrhosis, 1.38 ± 0.11; primary hepatoma, 1.79 ± 0.25. Urinary excretions of cGMP are significantly increased in liver cirrhosis and in hepatoma patients compared to those of normal controls, acute hepatitis and chronic hepatitis cases ($p < 0.001$). The cGMP levels of the recovery cases of acute hepatitis* and those of Wilson's disease° were not different from those of the rest of the cases in respective groups.

hepatoma as shown in Fig. 2. The distribution of individual values in these two groups was also greater than in others as analyzed by the *F* test. Cirrhotic patients with increased urinary excretions of cGMP had more marked reduction in effective hepatic blood flow as revealed by a positive correlation between cGMP excretion and K_{ICG} (disappearance rate constant for indocyanine green). Furthermore, significant correlations were also found between cGMP excretion and serum levels of cholesterol, protein, GOT/GPT ratio and cholinesterase activity, indicating the presence of more advanced hepatic injuries in those cirrhotic patients. No such correlations except for K_{ICG} were found in hepatoma patients (Table 1). In other words, the level of cGMP excretion in primary hepatoma was determined independent of the severity of parenchymal injury of the underlying cirrhotic lesion. Since the daily excretions of cGMP in individual hepatoma patients did not vary significantly during the course of illness (the

TABLE 1. CORRELATIONS BETWEEN URINARY EXCRETIONS OF cGMP AND RESULTS OF VARIOUS LIVER FUNCTION TESTS

Liver function test	LC		PH		LD	
	r	N	r	N	r	N
S-GOT	−0.118	27	0.049	19	−0.226*	78
S-GPT	−0.115	27	0.034	19	−0.228*	78
S-GOT/S-GPT ratio	0.380*	27	0.207	19	0.440**	76
S-Cholinesterase	−0.474*	25	0.064	17	−0.470**	73
S-Protein	−0.466*	26	0.045	19	−0.198	77
Alb/Glob	−0.155	23	−0.231	19	−0.500**	74
S-Cholesterol	−0.480*	25	−0.013	19	−0.146	76
S-ALP	−0.008	22	−0.072	18	−0.019	69
S-LAP	−0.011	24	−0.129	19	−0.036	70
S-LDH	0.504*	19	−0.066	15	−0.074	62
S-GGT	−0.069	23	−0.143	19	−0.008	72
K_{ICG}	−0.504**	26	−0.572*	15	−0.617**	64
S-Bilirubin (total)	0.452*	26	−0.109	19	−0.142	77
FBS	0.146	17	0.340	10	0.289	43
S-Calcium	0.014	12	−0.114	12	−0.141	41
AFP	−0.378	13	0.187	10	0.098	27
S-γ-globulin	−0.073	24	−0.302	16	0.085	71

Abbreviations used are: LC, liver cirrhosis; PH, primary hepatoma; LD, liver diseases including acute and chronic hepatitis, liver cirrhosis and primary hepatoma; N, number of cases; S, serum; r, correlation coefficient; GOT, aspartate aminotransferase; GPT, alanine aminotransferase; Alb, albumin; Glob, globulin; ALP, alkaline phosphatase; LAP, leucine aminopeptidase; LDH, lactate dehydrogenase; GGT, γ-glutamyl transpeptidase; FBS, fasting blood sugar; AFP, α-fetoprotein. *$p < 0.05$, **$p < 0.01$.

coefficient of variation of these cases being 20-30%), the extent of the increase in cGMP excretion appeared to be determined by the presence of hepatoma. However, the cGMP excretion had no prognostic significance in hepatoma patients. As a result of the increased cGMP excretion and the unchanged cAMP level in serum of hepatoma patients and the increased values of both cGMP and cAMP in liver cirrhosis, the ratio of cGMP excretion to plasma cAMP level clearly differentiated the hepatoma from liver cirrhosis (Fig. 3).

In order to see whether or not the increased plasma cAMP concentration has any relation to the presumed elevation of plasma glucagon in liver diseases, plasma levels of glucagon in patients with acute hepatitis and liver cirrhosis were determined and compared with those of cAMP. The plasma glucagon level was higher both in acute hepatitis (369 ± 66 pg/ml, $n = 9$, 164-717 pg/ml) and liver cirrhosis (375 ± 52 pg/ml, $n = 6$, 207-523 pg/ml), compared with 40-160 pg/ml in normal controls, but it had no significant correlation with the cAMP level ($r = -0.224$, $p > 0.05$).

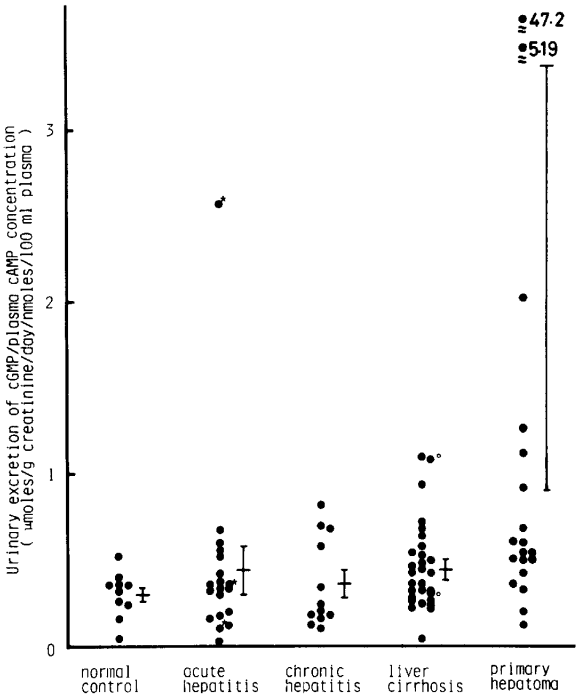

Fig. 3. The ratios of urinary excretion of cGMP to plasma cAMP level in patients with liver diseases on admission or on first determination. Mean and SEM are: normal controls, 0.30 ± 0.04; acute hepatitis, 0.44 ± 0.13; chronic hepatitis, 0.35 ± 0.07; liver cirrhosis, 0.44 ± 0.05; primary hepatoma, 3.34 ± 2.45. Significance levels of differences in the ratios between primary hepatoma and other groups are: $p < 0.01$ for acute hepatitis, $p < 0.05$ for chronic hepatitis and liver cirrhosis and $p < 0.01$ for normal controls. Recovery cases of acute hepatitis and Wilson's disease cases were indicated with the same marks as in Figs. 1 and 2.

TABLE 2. CONCENTRATIONS OF PLASMA cAMP AND cGMP

Case	Age	Sex	DX	Plasma cAMP levels (nmoles/100 ml)					
				SPV	SMV	POV	PEV	PEA	HPV
1	57	F	LC	2.75	2.75	2.58	2.65		
2	45	M	LC	3.80	4.10	3.25	4.25		
3	64	M	LC	5.40	4.30	4.80	3.85		
4	59	F	LC	5.30	5.30	5.55	5.40		
5	36	M	LC	5.75	5.00	4.70	5.30		
6	61	F	LC	2.52	3.10	2.75	2.50	2.14	2.42
7	46	M	LC	5.30	6.30	5.25	4.60		
8	51	M	LC	3.20	3.50	3.20	3.40	2.91	3.20
9	70	M	LC	4.50	4.25	3.70	4.20		
10	65	M	LC	4.40	3.99	4.30	2.45		2.15
11	27	M	NS	3.62	4.90	5.30	4.95		4.10

TABLE 2. continued

Case	Age	Sex	DX	Plasma cGMP levels (pmoles/100 ml)					
				SPV	SMV	POV	PEV	PEA	HPV
1	57	F	LC	5.6	8.0	8.1	7.4		
2	45	M	LC	13.3	15.8	17.7	16.5		
3	64	M	LC	17.0	14.1	14.1	10.4		
4	59	F	LC	9.8	17.4	30.2	29.8		
5	36	M	LC	7.6	16.6	11.4	7.9		
6	61	F	LC	11.3	13.4	12.1	12.2	11.6	6.4
7	46	M	LC	23.6	34.8	38.5	22.2		
8	51	M	LC	8.9	13.5	12.7	14.3	11.6	6.9
9	70	M	LC	8.0	15.5	13.3	18.8		
10	65	M	LC	14.2	13.3	12.2	8.6		7.3
11	27	M	NS	5.5	11.2	10.0	8.7		7.0

Abbreviations used are: DX, diagnosis; LC, liver cirrhosis; NS, no specific liver disorder with splenomegaly in the past; SPV, splenic vein; SMV, superior mesenteric vein; POV, portal vein; PEV, peripheral vein (cubital); PEA, peripheral artery (cubital); HPV, hepatic vein.

Plasma cAMP and cGMP concentrations determined on peripheral, portal system and hepatic venous blood samples are presented in Table 2. No significant differences in cAMP level were found among different sites of blood withdrawal, indicating that there were no principal organs producing or taking up the nucleotide. On the other hand, the cGMP level was generally lower in the splenic vein than in the superior mesenteric or the portal vein, and lowest in the hepatic vein, showing the importance of the liver as an organ removing the cGMP produced in the gut. However, the difference in cGMP concentration between the portal and hepatic veins could not be related to the peripheral concentration of cGMP in the present analysis on the limited number of cases studied.

DISCUSSION

Neethling reported increased excretions of urinary cGMP in three hepatoma cases in 1976 (9). Similar studies have been made but only on a limited number of hepatoma cases (18, 19). The increased excretion of cGMP in primary hepatoma was confirmed in the present study with a greater number of hepatoma cases. In addition, the increased excretion of cGMP was also found in liver cirrhosis. In interpreting the results, it should be noted first that all the primary hepatoma patients had liver cirrhosis. Therefore, the increased urinary excretion of cGMP is most likely to be ascribed to the common underlying disease, liver cirrhosis.

In experimental animals, the gut is considered to be a major site of cGMP

production and the liver to be an important organ for its removal (20, 21). This also applies to the metabolism of cGMP in humans as evidenced by the results of the present study with catheterization; *i.e.* higher concentrations of plasma cGMP were found in the portal and superior mesenteric veins and the lowest in the hepatic vein. Thus, the liver takes up a significant proportion of cGMP produced in the gut. The reduced uptake of cGMP by the liver may be reflected by the increase in peripheral venous level of cGMP, and hence the increased urinary excretion (19, 22). Increased plasma cGMP levels in hepatoma as well as liver cirrhosis have also been reported (19, 23). Since K_{ICG} represents a measure of effective hepatic blood flow (24) and it correlated with the increased urinary excretion of cGMP in patients with liver cirrhosis and primary hepatoma, the increased cGMP excretion could be explained by a reduced uptake of cGMP. In addition to the decreased perfusion of the cirrhotic liver, parenchymal liver injury appears to be involved in the reduced uptake of cGMP since significant correlations also existed between the urinary excretion of cGMP and the results of other liver function tests in patients with liver cirrhosis. No such correlation was found in primary hepatoma, even though liver cirrhosis coexisted. This is probably because the presumed production of cGMP by the hepatoma tissue (5, 7) overshadowed the effect of liver cirrhosis in reducing the uptake of cGMP. No further evidence to support this view was obtained in the present study. It is mere conjecture that the cirrhotic liver, which is considered to be a preneoplastic state, also produces an increased amount of cGMP. Wood and others (22) recently reported the increase in concentration of extracellular fluid cGMP in patients with liver cirrhosis, its levels being comparable to those with malignant disease, although Chawla *et al.* described negative results (25).

In contrast with the increased cGMP excretion in urine of both cirrhotic and hepatoma patients, the plasma level of cAMP was elevated only in liver cirrhosis, even though all the hepatoma patients had liver cirrhosis as an underlying hepatic lesion. Thus, the increased ratio of urinary cGMP excretion to the plasma cAMP level effectively differentiated primary hepatoma from liver cirrhosis as reported in other malignant diseases (18, 22). Since the urinary excretion of cAMP is affected more by the kidney function related to parathyroid hormone (26), the ratio of urinary cGMP to plasma cAMP employed in our present study is more rational than the ratio of urinary cGMP/urinary cAMP level in assessment of the disease (18). The increased plasma level of cAMP in liver cirrhosis could not be explained on the basis of increased plasma glucagon in the present study or norepinephrine in others (27). Therefore, it might well be a reflection of the preneoplastic state as is observed in DL-ethionine- and 3'-methyl-4-dimethyl-aminoazobenzene-induced hepatocarcinogenesis in rat (5, 6).

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