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CYCLIC AMP METABOLISM AND ADENYLATE CYCLASE CONCENTRATION IN PATIENTS WITH ADVANCED HEPATIC CIRRHOSIS

ANTONIO FRANCAVILLA, M.D., ARTHUR F. JONES, M.D., AND THOMAS E. STARZL, M.D., PH.D.

Department of Surgery, Denver Veterans Administration Hospital and the University of Colorado Medical Center, Denver, Colorado; and The Department of Gastroenterology, University of Bari, Bari, Italy

Glucagon was tested for its effect on plasma adenosine 3′,5′-cyclic monophosphate (cyclic AMP), insulin, and glucose in healthy subjects and in patients with advanced cirrhosis of the liver. In the normal subjects, intravenous infusion of glucagon caused a significant increase in plasma cyclic AMP, glucose, and insulin. In advanced cirrhotics, plasma cyclic AMP, glucose, and insulin did not increase. Adenylate cyclase concentration was measured in liver tissue from end stage cirrhotic patients and from brain-dead organ donors whose cardiovascular function was maintained in a stable state. Basal and total adenylate cyclase concentration were not different in the two groups. Adenylate cyclase from the livers of advanced cirrhotics was, however, significantly less responsive to glucagon stimulation than was that from donor livers. Hepatocytes in advanced cirrhosis have abnormal metabolic behavior characterized by abnormal adenylate cyclase-cyclic AMP response to hormonal stimulation.

Intracellular effects mediated by adenosine 3′,5′-cyclic monophosphate (cyclic AMP) have been described after interaction of many hormones with their target tissues.[1] Several studies have shown that cyclic AMP can pass from the intracellular compartment into plasma[2–4] and that the rate of this passage can be altered by changes in plasma concentrations of certain hormones.[5–8] Changes in rate of cyclic AMP passage might be attributable to changes in intracellular nucleotide concentration or in cell membrane permeability.[9]

Intravenous infusion of glucagon into normal humans has been shown to increase plasma and urine concentrations of cyclic AMP.[10] Liljenquist et al. have indicated that the liver is an important source of circulating cyclic AMP in man, and that glucagon increases the release of this nucleotide into the hepatic vein.[11] Recent studies by Strange and Mjøs on functionally hepatectomized rats have shown that the liver is the main source of glucagon-stimulated increase in plasma cyclic AMP.[12] Confirmatory data from rabbits have been reported by Jerums et al.[13]

Because cyclic AMP is known to stimulate glycogenolysis, gluconeogenesis, and lipolysis in the hepatic cell,[14–17] the "glucagon test" measuring blood-glucose response to glucagon injection has been proposed as an index of liver function.[18–20] Results have been contradictory, however, in large part because glucose metabolism in cirrhosis is affected by many factors.[21–26]

We have remained convinced that changes in plasma cyclic AMP levels in response to glucagon infusion may be of great potential interest as a test of the metabolic status and/or functional capability of hepatic cells. However, recent reports[27, 28] have not agreed on the effects of glucagon infusion in cirrhotic patients. The present study was undertaken to examine the effects of exogenous glucagon infusion on plasma cyclic AMP and liver adenylate cyclase concentration and on insulin and glucose levels in control subjects and in patients with decompensated liver cirrhosis.

Because hyperglucagonemia has been demonstrated in cirrhotic patients[29, 30] and has been shown to affect responsiveness of adenylate cyclase to glucagon stimulation,[31–34] we have also felt that studying hepatic adenylate cyclase concentration in this situation was important.

Patients and Methods

Informed consent was obtained from all subjects before inclusion in the study.

Subjects

Glucagon infusion tests were performed on 9 healthy adult subjects and on 11 patients with advanced liver cirrhosis. The diagnosis of liver cirrhosis was made on clinical grounds and supported by liver function tests and liver biopsy.

A complete liver profile including determination of serum bilirubin, alkaline phosphatase, SGOT, SGPT, serum choles-

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Address requests for reprints to: Thomas E. Starzl, M.D., Ph.D., Department of Surgery (C-305), University of Colorado Medical Center, 4200 East Ninth Avenue, Denver, Colorado 80262.

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terol, serum total protein and protein electrophoresis, blood ammonia and serum bromsulphalein retention, and prothrombin time was carried out in all patients before glucagon infusion. Liver function tests in these cirrhotic patients are listed in table 1. All control patients had liver profiles within the normal range.

Liver biopsy was done in cirrhotics a few minutes before the infusion test whenever possible (prothrombin time up to 18 sec with control of 12 sec). In any case, all cirrhotic patients had liver biopsies confirming cirrhosis at some time before inclusion in the study. Both ascites and esophageal varices demonstrated by barium esophagogram were present in all advanced cirrhotics none of whom had undergone gastric or portal surgery.

In addition, adenylate cyclase concentration was determined on liver tissue from 9 more patients with advanced cirrhosis at the time of liver transplantation. Liver profiles from these patients are shown in table 1. Cirrhotic liver adenylate cyclase was compared with liver adenylate cyclase concentration from 5 previously healthy brain-dead organ donors with stable cardiovascular function.

Experimental Procedures

Glucagon infusion tests were performed after an overnight fast. All patients had peripheral venous catheters placed and 0.9% saline infused. Control venous blood samples for plasma cyclic AMP, insulin, and glucose were drawn with heparinized syringes 20 and 10 min before glucagon infusion. A solution of glucagon (synthesized by Nova Industrials Laboratory, Denmark) in 0.9% saline was substituted for the saline and infusion continued at a rate of 200 ng of glucagon per kg per min by means of a Dascon infusion pump (BV Dascon, Uden, Netherlands). Peripheral venous blood samples were obtained with heparinized syringes at 10-min intervals for 60 min. Plasma was immediately separated by centrifugation (3 to 5°C) and stored at −20°C until analysis.

Liver taken from cirrhotic patients before liver transplantation and from brain-dead donors was immediately cooled in ice-cold saline and assayed.

Analytical Procedures

Cyclic AMP. Cyclic AMP was determined by the protein binding assay of Gilman.[35] Cyclic AMP-dependent protein kinase was purified from 500 g of sirloin tip steak by the method of Miyamoto et al.[36] Protein kinase inhibitor preparation was carried out according to Walsh et al.[37] Nonradioactive cyclic AMP was obtained from Sigma Chemical Company (St. Louis, Mo.), tritiated [8-^{3}H]adenosine 3′,5′-cyclic monophosphate with specific activity of 28 Ci/m mol from Schwarz/Mann, Inc. (Orangeburg, N. Y.), and cellulose ester filters (HA 0.45 mm) from Millipore Corporation (Bedford, Mass.).

After deproteinization with ethanol, plasma samples for cyclic AMP assay were centrifuged and the supernatant evaporated to dryness under nitrogen stream at 55°C. The residue was then dissolved in 0.5 ml of 0.2% sodium acetate buffer (*p*H 4.0) and reaction was conducted in a volume of 250 μl. Cyclic nucleotide was subsequently measured and corrected for the recovery of [^{3}H]cyclic AMP. To confirm that cyclic AMP had been measured, the samples were treated with cyclic AMP-phosphodiesterase as described by Brooker et al.[38] No cyclic AMP could be detected after this treatment.

Plasma insulin was determined by radioimmunoassay using the insulin kit from CIS (Sorin, Saluggia based on the method of Yalow and Berson[39]).

Glucose. Plasma glucose was determined with a commercial glucose oxidase preparation (Biochemica Test Combination, Boehringer Mannheim GmbH, Mannheim, West Germany). All plasma samples were measured in duplicate.

Adenylate cyclase. Homogenate from human livers was prepared immediately after biopsy, according to the method of Makman and Sutherland.[40] Small pieces of liver were forced through a fine mesh metal sieve with a hand-operated flut plunger before homogenization with a Potter-Elvehjem homogenizer. The homogenate was then rapidly frozen in liquid nitrogen and stored until use.

Basal, glucagon-stimulated, and fluoride-stimulated adenylate cyclase concentrations were determined by the method of Salomon et al.[41] based on a combination of chromatography

TABLE 1. *Liver function tests in cirrhotic patients*

Group		Total protein	Albumin	γ-Globulin	Serum cholesterol	Blood ammonia	BSP[a] retention	SGOT	SGPT	Alkaline phosphatase	Total bilirubin	PT[b]
	U	*g/100 ml*	%	%	*mg/100 ml*	*μg/100 ml*	%	*mU/ml*		*IU*	*mg/100 ml*	*sec*
Normal		6.6–8.7	55–68	14–19	150–250	21–86	<4.7	<12	<12	25–92	<1.0	<12.0
Glucagon infusion test subjects	1	5.4	41	40	100	150	18	20	15	35	1.53	14
	2	6.0	45	43	130	240	43	18	15	170	1.26	16
	3	5.1	38	47	40	160	40	22	10	608	6.40	21
	4	5.0	40	50	130	300	68	25	12	325	1.00	18
	5	5.6	45	32	120	350	32	32	13	424	8.60	20
	6	4.6	44	38	83	180	42	57	59	191	1.05	23
	7	5.0	47	42	103	220	38	21	5	56	2.76	16
	8	5.2	50	30	100	160	41	30	10	74	18.70	19
	9	6.0	35	47	98	200	20	92	77	134	7.00	14
	10	5.4	48	30	105	160	25	80	74	105	6.50	15
	11	6.1	45	35	90	140	30	50	40	80	2.50	18
Liver adenylate cyclase subjects	1	6.0	30					273	132	274	13	14
	2	7.1	41					93	60	1270	7	12
	3	6.8	51					92	51	450	6.9	11
	4	3.9						142	375	572	2.5	14.5
	5	5.2	35					208	134	390	18	15
	6	5.3	43					335	155	726	21	12
	7	6.1	43					39	59	41	39	21
	8	5	50					17	15	116	1.5	13
	9	7.5	60					334	215	2055	22.8	15

[a] BSP, bromsulphalein.
[b] PT, prothrombin time.

on both Dowexcation exchange resin according to Krishna et al.[42] and aluminum oxide according to White and Zenser[43] as previously described.[44]

Statistical procedures. The mean of values obtained at 10 and 20 min before glucagon infusion was considered as the control value for plasma cyclic AMP, insulin, and glucose.

All results have been expressed as mean ± SEM. Differences between values were tested for significance by Student's *t*-test.

Results

Glucagon infusion tests. Table 2 shows plasma cyclic AMP concentration in healthy subjects and cirrhotic patients during infusion of 0.9% saline at 20 and 10 min before glucagon infusion. In healthy subjects, plasma cyclic AMP rose promptly and remained significantly elevated throughout the glucagon infusion test. In contrast, cyclic AMP failed to rise in advanced cirrhotics (fig. 1).

In healthy subjects, insulin concentrations rose significantly and remained elevated throughout the glucagon infusion test. In cirrhotics, only minimal insignificant increases occurred (fig. 2).

TABLE 2. *Basal plasma cyclic AMP in healthy subjects and in patients with advanced cirrhosis*

	Saline infusion	
	20 min[a]	10 min[a]
	pmole/ml	
Healthy subjects (n = 9)		
Range	6–23	0–25
Mean	13.55	15.88
±SEM	1.84	2.34
Cirrhotic patients (n = 11)		
Range	6–18	7–16
Mean	12.54	11.81
±SEM	1.31	1.06

[a] Time before glucagon infusion.

Plasma glucose levels also rose rapidly and significantly in healthy subjects. Although base-line plasma glucose levels were significantly higher in cirrhotics than in healthy subjects, plasma glucose did not increase in response to glucagon infusion in these patients (fig. 3).

Hepatic adenylate cyclase concentration. Basal, glucagon-stimulated, and sodium fluoride-stimulated activities from donor livers are reported in table 3. Glucagon-stimulated activities increased steadily with increasing glucagon concentrations until near total (sodium fluoride-stimulated) activity was achieved at glucagon concentration of 10^{-5} M.

Adenylate cyclase concentrations from end stage cirrhotic livers were more heterogeneous (table 3). Although basal and total concentrations did not differ significantly from those of donor livers, they seemed to fall into high activity and low activity groups. In these cirrhotic livers, glucagon-stimulated concentrations rose less rapidly and to significantly lower levels with increasing glucagon concentration. Near total stimulation was never achieved with glucagon stimulation.

Discussion

Our results indicate that intravenous glucagon infusion causes different responses in healthy subjects and in patients with advanced cirrhosis. In normal subjects, glucagon infusion caused a rapid increase in both plasma cyclic AMP levels and plasma glucose. These increases were expected because it has been demonstrated that the effect of glucagon on the liver depends on an adenylate cyclase-mediated increase in cyclic AMP.[14–17] Both plasma cyclic AMP and plasma glucose then plateaued with continued infusion of glucagon. Plasma insulin had substantially increased by that time. The effects on insulin have been attributed to glucagon administration,[45] hyperglycemia,[46–48] and in-

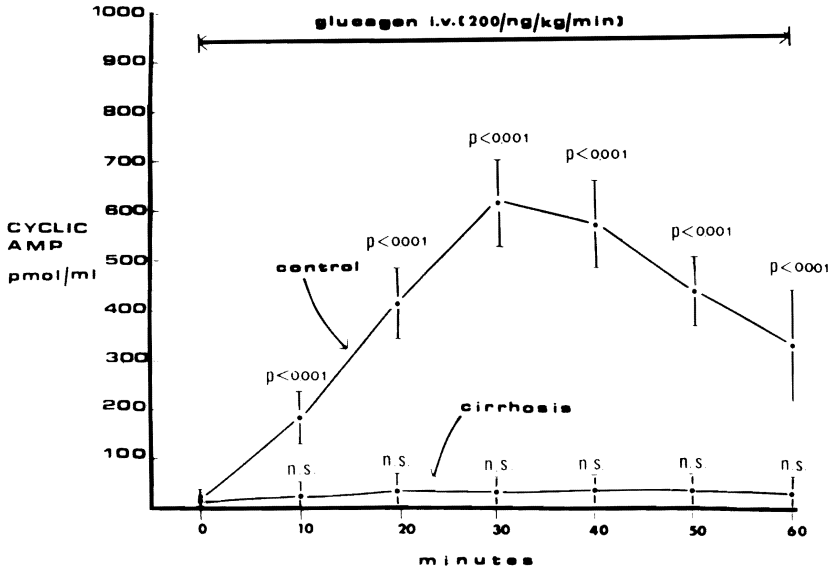

FIG. 1. Plasma cyclic AMP concentration in 9 control subjects and in 11 cirrhotic patients at various intervals during glucagon infusion. Values are mean ± SEM and show the probability that concentrations during fasting do not differ from those at zero time (paired Student's *t*-test); *n.s.*, not significant.

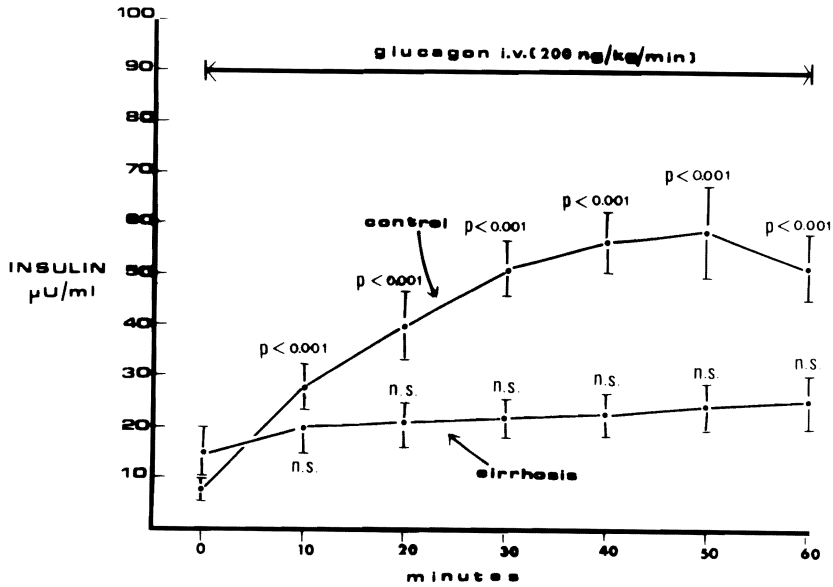

FIG. 2. Plasma insulin concentration in 9 control subjects and in 11 cirrhotic patients at various intervals during glucagon infusion. Values are mean ± SEM and show the probability that concentrations during fasting do not differ from those at zero time (paired Student's *t*-test); *n.s.*, not significant.

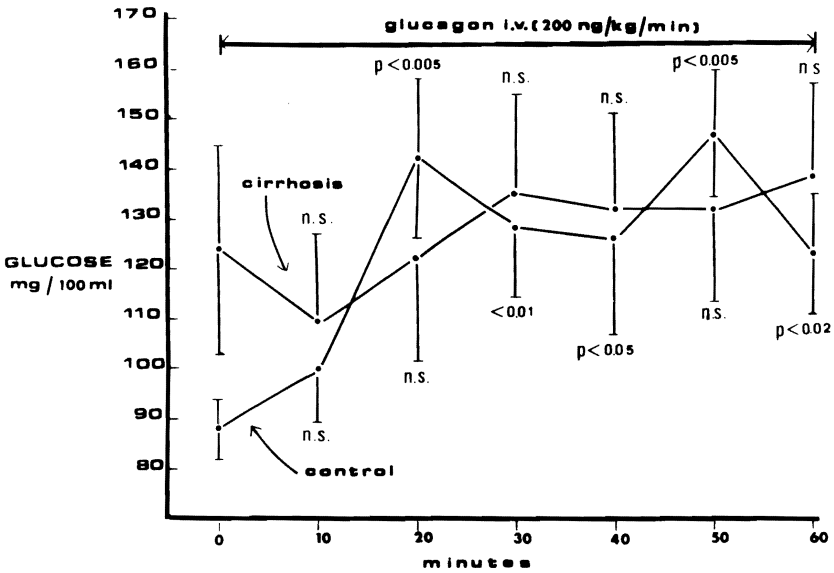

FIG. 3. Plasma glucose concentration in 9 control subjects and 11 cirrhotic patients at various intervals during glucagon infusion. Values are mean ± SEM and show the probability that concentrations during fasting do not differ from those at zero time (paired Student's *t*-test); *n.s.*, not significant.

creases in circulating cyclic AMP.[49–53] The increase in insulin levels could account for the transient nature of the glucagon mediated effect because insulin has been demonstrated to decrease hepatic cyclic AMP and the glycogenolytic and gluconeogenic effect of cyclic AMP. This antagonism between insulin and glucagon represents one of the most important factors controlling liver metabolism.[54–57] Wahren et al. have demonstrated, however, that the effect of glucagon on splanchnic glucose production is evanescent even in the face of absolute insulin deficiency.[58]

On the other hand, plasma cyclic AMP, glucose, and insulin all failed to respond to glucagon infusion in the patients with advanced cirrhosis. This unresponsiveness might be attributed to several factors. Glucagon might bypass the liver by way of portal systemic shunts because all of these patients had demonstrated esophageal varices. This is probably not of major importance in our studies because infusion times were long and hormone doses high. Impaired responsiveness of hepatic adenylate cyclase has been demonstrated in hyperglucagonemic states from either endogenous or exogenous glucagon.[31–34] We have demonstrated decreased responsiveness of hepatic adenylate cyclase to glucagon stimulation in our group of end stage cirrhotic patients submitted to liver transplantation. Although basal and

TABLE 3. *Adenylate cyclase concentration in donor and end stage cirrhotic livers (nanomoles cyclic AMP/mg protein homogenate/15 min)*[a]

No.	Diagnosis	Age	Basal H_2O	Glucagon stimulated					NaF stimulated (10 mM)
				10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	
		yr							
Donor									
1			50.8	132	167	234	226	269	273
2			80.7	109	121	161	218	331	336
3			80	105	152	232	293	329	304
4			65	87	124	203	212	248	289
5			51	64	77.4	109	148	129	209
Mean ± SD			65.5 ± 14.7	99.3 ± 22.5	132 ± 30.8	188 ± 47.7	230 ± 55.8	270 ± 59.1	282 ± 47
Cirrhotic									
1	Biliary atresia	10 mo	49.1	49.5	51.5	54.0	54.0	59.4	201
2	Arteriovenous malformation	51	41.2	62.2	66.3	72.9	82.4	76.2	135
3	Biliary atresia	11	37.1	47.7	54.5	60.4	59.3	61.2	154
4	α_1-Antitrypsin deficiency	5 1/2	43.8	61.7	74.0	91.5	99.4	112	197
5	Biliary atresia	3 1/2	165	184	219	287	288	282	519
6	Chronic aggressive hepatitis	14	94	98	125	198	212	207	441
7	Alcoholic cirrhosis	35	55	55	97	95.7	113	128	200
8	Alcoholic cirrhosis	46	162	164	168	202	194	185	583
9	Biliary atresia	13 mo	57.9	67	66	84.5	90.8	109	215
Mean ± SD			75 ± 47	90 ± 50	102 ± 54.5	127 ± 76	133 ± 26	136 ± 70	306 ± 167
P[b]			NS[c]	NS	NS	NS	<0.02	<0.01	NS

[a] Basal activity reflects receptor and catalytic components of adenylate cyclase concentration; glucagon-stimulated values reflect receptor activity at different molar concentrations of glucagon; sodium fluoride (NaF)-stimulated values reflect the full (catalytic) concentration of adenylate cyclase.

[b] Compared to donor liver by Student's *t*-test.

[c] NS, not significant.

total adenylate cyclase concentrations were not different from those determined for nondiseased donor livers, glucagon stimulation was much less effective and failed to approach maximal response in the livers from these end stage cirrhotic and, no doubt, hyperglucagonemic patients. The mechanism for this impairment is speculative. Srikant et al. have recently demonstrated a reduction in glucagon-binding sites in livers of rats made chronically hyperglucagonemic.[59] Rather surprisingly, these investigators were unable to show a correlation between terminal plasma glucagon concentration and the reduction in receptor concentrations. In their hyperglucagonemic models, the ability of glucagon to stimulate adenylate cyclase was not reduced proportionately by the reduction of glucagon-binding sites.

We can only speculate on why our patients with cirrhosis responded significantly differently to glucagon infusion from those reported by Strange et al.,[28] who found no significant difference in cyclic AMP or glucose response between cirrhotics and controls. We employed higher doses of glucagon and achieved much higher plasma cyclic AMP levels in our normal controls. A more likely explanation is that our patients had more heterogeneous cirrhosis than did their generally well compensated patients. Our patients, with demonstrated spontaneous portal systemic shunting, responded much more like the cirrhotics who had undergone shunt surgery reported by Davies et al.[27] Our results suggest that hepatocytes from patients with advanced cirrhosis have abnormal metabolic behavior or, more specifically, an abnormal adenylate cyclase system. This unresponsiveness to known hepatotrophic factors[57] could further damage the already injured liver.

These findings suggest new prospects for the glucagon infusion test in determining the degree of parenchymal dysfunction in patients with liver disease and cirrhosis.

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