Relationship between Glucagon-Induced Cyclic AMP Accumulation and DNA Synthesis in Carbon Tetrachloride-Intoxicated Rat Liver

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It has been suggested that hepatocyte proliferation is controlled by humoral factors (1). Glucagon, in particular, has been said to be involved in the proliferation of liver cells in experiments using intact livers (2), regenerating livers after partial hepatectomy (3), and cultured liver cells (4). The growth-promoting effect of glucagon may be mediated via cyclic AMP since cyclic AMP can effectively mimic glucagon in the stimulation of hepatic DNA synthesis (4, 5). On the other hand, little work has been done to study the effect of glucagon on the regeneration of hepatocytes following liver injury (6, 7), and the role of glucagon in the regeneration of injured liver is not well defined.

The present study was undertaken to examine whether or not glucagon is involved in the regeneration of injured liver. The effect of glucagon on hepatic DNA synthesis and the relationship of glucagon-induced hepatic accumulation of cyclic AMP to the glucagon effect on DNA synthesis were studied in carbon tetrachloride (CCl_4)-intoxicated rats.

Male Wistar rats (weighing 260–320 g) were used for the experiments. CCl_4 (1 ml/kg, b.w.) in 50% olive oil solution was administered by a stomach tube and normal control rats received olive oil alone. To determine hepatic DNA synthesis, rats were injected intraperitoneally with 5 μCi (methyl- ^3H)thymidine (25 Ci/mmol, Amersham) per 100 g body weight and killed 1 hr later. A portion of the liver (1 g) was homogenized in 19 volumes of cold distilled water. DNA was extracted from the homogenate as described by Munro and Fleck (8), and the amount of radioactivity incorporated into DNA was determined by counting on a Beckman LS 9000 scintillation spectrometer.

DNA was measured by the method of Martin et al. (9) using calf thymus DNA standards. ^3H -Thymidine incorporated into liver DNA was expressed as cpm/mg DNA. Because the peak of stimulated DNA synthesis was found 24 hr after the injection of the hormone in preliminary experiments, glucagon (500 $\mu\text{g/kg}$) (Sigma Chemical Co., St. Louis) or saline (as control) was injected subcutaneously 24 hr before the estimation of DNA synthesis.

To examine the effect of glucagon on liver cyclic AMP, the hepatic level of cyclic AMP was determined. Under pentobarbital anesthesia, rats were subcutaneously injected with glucagon (500 $\mu\text{g/kg}$) or saline. Fifteen min after the injection, a portion of the liver was excised and rapidly frozen in a liquid nitrogen-cooled clamp. Tissue content of the cyclic AMP was measured by radioimmunoassay (10) using a commercial assay kit (Yamasa, Choshi, Japan). The adenylate cyclase response to glucagon was also determined in normal and CCl_4 -treated rats. A portion of the liver was homogenized with ice-cold 25 mM Tris buffer, pH 7.6, containing 5 mM MgCl_2 and 1 mM dithiothreitol. The homogenate was filtered through four layers of cheesecloth and centrifuged at $900\times g$ for 5 min. The sediment was washed three times and resuspended in the same homogenization medium. The $900\times g$ particulate fractions were stored at -80°C until the analysis of the adenylate cyclase. The adenylate cyclase activity was measured using (α - ^{32}P) ATP (45 Ci/mmol, Amersham) according to the method of Salomon et al. (11) in the presence of 10 mM theophylline. Incubation was performed for 10 min at 30°C . The reaction was stopped with a solution of 2% sodium dodecyl sulfate, 40

mM ATP and 1.4 mM cyclic AMP. The sample was counted on a Beckman LS 9000 scintillation spectrometer. Protein was determined by the method of Lowry et al. (12). Results are given as the mean $\pm$ S.E. Student's *t*-test was used for calculating statistical significance.

The effects of glucagon on hepatic DNA synthesis in normal and CCl₄-treated rats are shown in Table 1. Basal DNA synthesis (without glucagon) was elevated 48 and 72 hr following CCl₄ intoxication, indicating liver regeneration after the intoxication (13), and the peak of basal DNA synthesis was observed at 48 hr. Hepatic DNA synthesis in normal rats was increased by glucagon to 2-fold that of basal DNA synthesis. This result is compatible with a previous report (2). Furthermore, 48 hr after CCl₄ intoxication in rats, glucagon strikingly stimulated hepatic DNA synthesis to 4.8-fold that of basal DNA synthesis. This stimulatory effect

of glucagon was also observed 72 hr after the intoxication. Thus, glucagon effectively stimulated DNA synthesis in rats after CCl₄ intoxication and marked stimulation of DNA synthesis by glucagon was observed coincidentally in time with the peak of elevation in basal DNA synthesis. These results indicate that glucagon plays a positive role in regulating liver regeneration after CCl₄ intoxication.

To examine the possible relationship between the effects of glucagon observed in Table 1 and the ability of glucagon to stimulate hepatic accumulation of cyclic AMP, the effects of glucagon in increasing the hepatic levels of cyclic AMP were studied in normal and CCl₄-treated rats. These results are shown in Table 2. Glucagon caused a remarkable increase of cyclic AMP in the livers of normal rats. On the other hand, hepatic accumulation of cyclic AMP by glucagon was significantly decreased both

Table 1. Effects of glucagon on hepatic DNA synthesis

Time after CCl ₄ administration (hr)	DNA synthesis (cpm/mg DNA)	
	Saline	Glucagon
0	2252 $\pm$ 115 (9)	4459 $\pm$ 451 (5) ^a
24	2681 $\pm$ 404 (3)	
48	5127 $\pm$ 563 (8) ^a	20724 $\pm$ 4339 (4) ^a
72	2894 $\pm$ 45 (4) ^a	4205 $\pm$ 508 (4) ^b

The results are given as the mean $\pm$ S.E. The number of observations is shown in parentheses. Glucagon (500 μ g/kg) or saline was injected 24 hr before the estimation of DNA synthesis. ^aP<0.05 compared with the saline-injected control in normal rats. ^bP<0.05, ^cP<0.001 compared with the corresponding saline-injected control.

Table 2. Effects of glucagon on hepatic levels of cyclic AMP and adenylate cyclase activity in the 900 $\times$ g particulate fractions

Time after CCl ₄ administration (hr)	cyclic AMP level (nmol/g tissue)		Adenylate cyclase activity (pmol cyclic AMP/mg protein/10 min)		
	Saline	Glucagon (500 μ g/kg)	None	Glucagon (10 ⁻⁶ M)	NaF (10 ⁻² M)
0	0.80 $\pm$ 0.07 (5)	9.13 $\pm$ 0.83 (3)	45.4 $\pm$ 8.3	477.0 $\pm$ 60.7	342.4 $\pm$ 32.3
24	0.84 $\pm$ 0.04 (4)	2.53 $\pm$ 0.54 (3) ^a	42.1 $\pm$ 7.5	283.2 $\pm$ 52.6 ^b	331.1 $\pm$ 29.4
48	1.03 $\pm$ 0.09 (8)	4.22 $\pm$ 0.33 (3) ^a	77.7 $\pm$ 11.8	336.8 $\pm$ 61.1	375.5 $\pm$ 82.1

The results are given as the mean $\pm$ S.E. The number of observations for hepatic cyclic AMP levels is shown in parentheses. The results of adenylate cyclase activity were obtained from 4 or 5 experiments in duplicate assay. ^aP<0.01 compared with the glucagon-induced accumulation of cyclic AMP in normal rats. ^bP<0.05 compared with the glucagon-stimulated activity in normal rats.

at 24 hr after the intoxication (to 28% of the glucagon-induced accumulation in normal rats) and at 48 hr (to 46%). Furthermore, consistent with the decline in the glucagon-induced hepatic accumulation of cyclic AMP, glucagon-stimulated activity of adenylate cyclase was found to be decreased in the 900×g particulate fractions obtained from rats 24 and 48 hr after the intoxication when compared with such activity in the particulate fractions obtained from normal rats (Table 2). In contrast, when normal and CCl₄-treated rats were compared, there was no appreciable alteration in fluoride-stimulated activity. A reduction in the adenylate cyclase response to glucagon in CCl₄-treated rats has been reported by Mourelle and Rubalcava (14). Since no essential difference in fluoride-stimulated activity of adenylate cyclase was observed between normal and CCl₄-treated rats (Table 2), the attenuation in the cyclic AMP response to glucagon observed in CCl₄-treated rats might partially result from a decrease in the number of glucagon receptors (14).

It has been suggested that the growth-promoting effect of glucagon is mediated by cyclic AMP. However, the potentiating effect of glucagon on hepatic DNA synthesis observed in CCl₄-treated rats could not be ascribed to the glucagon-induced hepatic accumulation of cyclic AMP. The apparent absence of any relationship between the effects of glucagon on cyclic AMP and DNA synthesis may suggest that additional factors other than cyclic AMP are also involved in the induction of hepatic DNA synthesis by glucagon. Laks et al. (15) recently reported that additional factors other than cyclic AMP are required for the increase of cyclic AMP-dependent protein kinase, which may be involved in the initiation of DNA synthesis (16), in the nucleus in the regenerating liver after partial hepatectomy. It may be possible that deficient states of insulin and iodothyronine, which occur before the initiation of DNA synthesis in CCl₄-treated rats (14, 17) and in partially hepatectomized rats (18), are responsible for the potentiating effect of glucagon on DNA synthesis. However, neither insulin deficiency nor iodothyronine deficiency, alone, brings about the potentia-

tion of the glucagon effect on DNA synthesis (17). Other interpretations may be possible and further investigations are needed to clarify the situation.

Although the mechanism of the potentiating effect of glucagon on hepatic DNA synthesis in CCl₄-treated rats is not clear, the present study suggests that glucagon participates in liver regeneration after CCl₄ intoxication.

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