Horm. metabol. Res. 19 (1987) 239–241

Hepatic Regeneration Following Glucagon Treatment

K.F. Petersen, B.A. Hansen and H.E. Poulsen

Divisions of Hepatology and Experimental Pathology, Rigshopitalet, Copenhagen, Denmark

Summary

Liver regeneration was studied as a function of time after suppression of the normal glucagon response. Rats were given a daily subcutaneous injection of 20 μg zincglucagon for 14 days, whereafter a 70% hepatectomy was performed.

In the glucagon treated rats the rise in plasma glucagon concentration was diminished after hepatectomy. At intervals from 12 to 384 hours after hepatectomy, the gain in liver weight, the hepatic DNA content, and the antipyrine clearance were measured. All 3 variables were found to be significantly higher in animals with diminished glucagon response.

The results indicate that prevention of the normal increase in glucagon concentration leads to signs of increased liver regeneration after 70% hepatectomy.

Key-Words: *Liver Growth – Glucagon – Liver Function*

Introduction

After partial hepatectomy plasma glucagon is increased (*Leffert, Koch, Morgan* and *Rubalcava* 1979) and this is assumed to be one of the stimuli to hepatic regeneration (*Bucher* and *Weir* 1976). In an earlier study we have found that the glucagon response to amino acid loading can be suppressed by long term administration of glucagon (*Petersen, Hansen* and *Vilstrup* 1987). The precise mechanism of this suppression is unknown, but atrophy of the pancreatic A-cells reported to be a consequence of administration of exogenous glucagon (*Logothetopoulos, Sharma, Salter* and *Best* 1960) is a possibility.

The purpose of the present work was to study the regeneration in rat liver after 70% hepatectomy during inhibition of the glucagon response in order to elucidate the role of glucagon in this situation.

Material and Methods

Female Wistar rats of 200 g were kept in cages of four with free access to stock rat pellets and tap water, at a constant temperature and a fixed 12 hour controlled light/dark cycle. The body weight was determined daily. For a period of 14 days a group of 36 rats was given a daily subcutaneous injection of 20 μg zincglucagon (NOVO, Denmark), 36 control rats were given 20 μg of the zinc vehicle. During this period the body weight in the two groups increased equally ($P > 0.05$, two way analysis of variance). Twenty-four hours after the last injection, a 70% hepatectomy was performed under diethylether anesthesia (*Higgens* and *Anderson* 1931). Five hours before the operation the animals received 4 mg (4 mg/ml) of antipyrine by stomach tube, for determination of antipyrine clearance (*Pilsgaard* and *Poulsen* 1984). Twelve hours after the hepatectomy four glucagon treated and four control rats were anesthetized with diethylether, the liver was removed, and the liver weight determined. Blood was collected from the aorta. A sample of heparinized blood was taken for estimation of the antipyrine plasma concentration (*Pilsgaard* and *Poulsen* 1984). The plasma glucagon and insulin concentrations were measured by RIA from trasylol-EDTA stabilized blood (*Heding* 1977). The DNA content of the liver was determined as described by *Munro* and *Fleck* (1966). This procedure was repeated after 18, 24, 30, 36, 48, 72, 170 and 384 hours.

Received: 19 Apr. 1985 **Accepted: 9 Aug. 1986 after revision**

Statistical Analysis

Differences between groups were assessed by Student's t-test, and among groups by two way analysis of variance; P-values < 0.05 were considered significant.

Results

The plasma glucagon concentration as a function of time is shown in Fig. 1a and Table 1. In the control group the glucagon concentration is increased by a factor 3 at 12 hours, followed by a further increase with a maximum at about 30 hours. The glucagon concentration is doubled from time 0 to 12 hours in the glucagon group; and is not increased after this. There is no difference in the plasma insulin concentration between the groups ($P > 0.05$).

At 30 hours the DNA content in the glucagon group significantly exceeds that of the controls (Fig. 1b and Table 2), and remains higher for the rest of the observation period ($P < 0.05$). Fig. 1c and Table 2 show that the change in antipyrine clearance with time shows the same pattern ($P < 0.05$). Twelve hours after hepatectomy the liver weight in the control group is slightly, but not significantly higher than in the glucagon group, but thereafter this is reversed ($P < 0.05$).

Discussion

In this study the increase in liver weight, DNA content, and antipyrine clearance after 70% hepatectomy is accelerated in rats given zincglucagon daily for 14 days.

As earlier reported (*Petersen* et al. 1987) the basal glucagon concentration in rats treated with zincglucagon is not different from the level in normal rats (*Hansen* and *Poulsen*

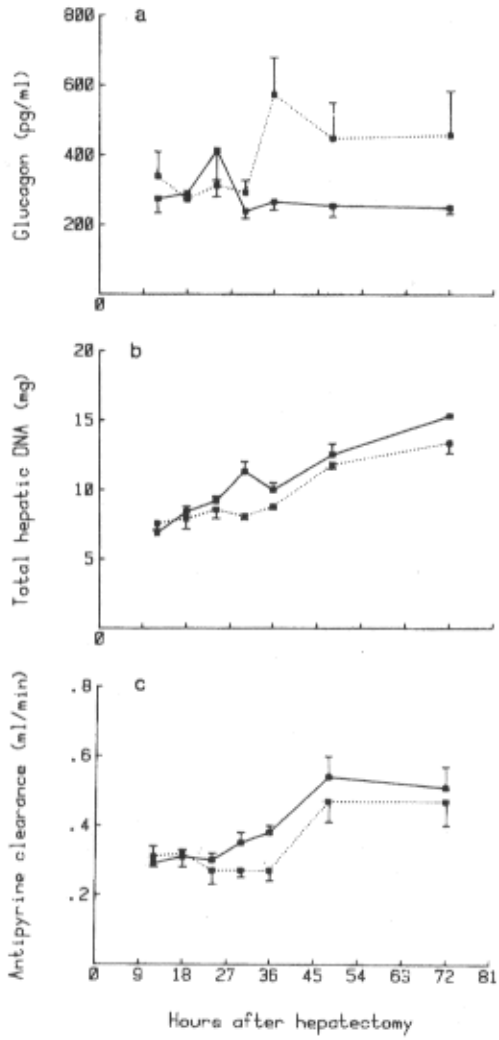

Fig. 1 Relation between plasma glucagon concentration (a), total hepatic DNA (b), antipyrine clearance (c), and time after 70% partial hepatectomy. Solid lines indicate glucagon pretreated and dotted lines control rats. Symbols indicate mean values and bars SEM of four animals.

1986). Twelve hours after the hepatectomy there is an increase in both groups; most pronounced in the control group. Thereafter there is a further increase with a maximum at about 30 hours in the controls; this is not seen in the glucagon group where the glucagon concentration remains at the 12 hour level.

The altered glucagon response in the glucagon group could be ascribed to either a glucagon feed-back inhibition or a damage of the pancreatic A-cells with some residual activity (*Logothetopoulos* et al. 1960).

The increase in the DNA content of the liver is assumed to reflect an increase in the amount of liver cells, and the increase in the antipyrine clearance an increase in the "functional liver mass" (*Poulsen* 1985). The earlier and more pronounced rise in both variables in rats given zincglucagon, indicates accelerated liver regeneration in these animals.

Table 1 Glucagon and insulin concentration at various intervals after 70% hepatectomy in control animals (C) and in animals treated with glucagon (T). Values are mean and SEM of four rats. n.d.: not determined.

Time interval (h)		Glucagon (pg/ml)	Insulin (uU/ml)
* 0	C	112 ± 20	18.8 ± 7.6
	T	137 ± 20	54.3 ± 26.0
12	C	339 ± 71	39.7 ± 8.5
	T	274 ± 40	28.5 ± 8.5
18	C	273 ± 11	44.5 ± 18.0
	T	290 ± 16	36.5 ± 13.0
24	C	313 ± 15	73.8 ± 22.0
	T	418 ±133	93.3 ± 39.0
30	C	293 ± 35	42.5 ± 6.4
	T	239 ± 21	53.3 ± 11.0
36	C	575 ±106	51.0 ± 7.3
	T	267 ± 24	63.9 ± 10.0
48	C	447 ±104	42.7 ± 15.0
	T	255 ± 32	40.0 ± 11.0
72	C	457 ±126	50.3 ± 24.0
	T	250 ± 18	36.8 ± 12.0
170	C	192 ± 16	23.5 ± 4.2
	T	207 ± 12	53.9 ± 3.5
384	C	255 ± 20	n.d.
	T	203 ± 16	n.d.

*Basal values as given in ref. (*Petersen* et al. 1987)

Table 2 Liver weight (LW), antipyrine clearance (APC), and total hepatic DNA at various intervals after 70% hepatectomy in control animals (C) and in animals treated with glucagon (T). Values are mean and SEM of four rats.

Time interval (h)		LW (g)	APC (ml/min)	DNA (mg)
12	C	3.62 ± 0.13	0.31 ± 0.03	7.57 ± 0.53
	T	3.34 ± 0.19	0.29 ± 0.05	6.86 ± 0.27
18	C	3.82 ± 0.22	0.32 ± 0.04	7.92 ± 0.75
	T	4.39 ± 0.15	0.31 ± 0.02	8.42 ± 0.38
24	C	4.31 ± 0.23	0.27 ± 0.04	8.57 ± 0.64
	T	4.69 ± 0.11	0.30 ± 0.02	9.19 ± 0.33
30	C	4.28 ± 0.15	0.27 ± 0.02	8.09 ± 0.26
	T	4.66 ± 0.22	0.35 ± 0.03	11.32 ± 0.70
36	C	4.62 ± 0.09	0.27 ± 0.03	8.79 ± 0.21
	T	4.83 ± 0.21	0.38 ± 0.02	9.98 ± 0.53
48	C	5.51 ± 0.41	0.47 ± 0.06	11.82 ± 0.30
	T	5.78 ± 0.56	0.54 ± 0.06	12.54 ± 0.79
72	C	6.40 ± 0.09	0.47 ± 0.07	13.41 ± 0.75
	T	6.87 ± 0.07	0.51 ± 0.06	15.34 ± 0.11
170	C	6.72 ± 0.44	0.49 ± 0.06	17.21 ± 1.00
	T	8.52 ± 0.78	0.48 ± 0.05	20.31 ± 1.55
384	C	7.07 ± 0.32	0.61 ± 0.04	18.61 ± 0.68
	T	7.81 ± 0.23	0.55 ± 0.07	17.76 ± 0.82

There is no difference in the insulin concentration after the hepatectomy between the two groups, and therefore insulin, also considered a "hepatotrofic factor" (*Caruana, Goldman, Camara* and *Gage* 1981), does not account for the difference in regeneration rate.

The zinc concentration in serum was not determined and as it is yet unknown if zinc has an effect in itself, the control group given the zinc-vehicle is not absolutely comparable.

This study suggests that the increase in glucagon after partial hepatectomy is an unspecific "catabolic" response which rather inhibits than enhances hepatic regeneration.

Acknowledgements

Our thanks are due to technicians Bjørg Krogh, Liselotte Hansen, Mie Poulsen, Inger Petersen, and Bodil Fick for expert laboratory assistance. NOVO, Denmark, supplied iodinated insulin, glucagon and antisera.

References

Bucher, N.L.R., G.C. Weir: Insulin, glucagon, liver regeneration, and DNA synthesis. Metabolism 25: 1423–1425 (1976)

Caruana, J.A., Goldman, J.K., Camara, D.S., A.A. Gage: Insulin glucagon and glucose in the regeneration response of the liver. Surgery 153: 726–730 (1981)

Hansen, B.A., H.E. Poulsen: Changes in the hepatic capacity of urea-N synthesis, galactose elimination, and antipyrine clearance following 70% hepatectomy in the rat. Scand. J. Clin. Lab. Invest. 46: 233–237 (1986)

Heding, L.G.: Radioimmunological determination of pancreatic and gut glucagon in plasma. Diabetologia 7: 10–19 (1977)

Higgens, G.M., R.M. Anderson: Experimental pathology of liver. I. Restoration of liver of white rat following surgical removal. Arch. Path. 12: 186–202 (1931)

Leffert, H.L., Koch, K.S., Morgan, T., B. Rubalcava: Hormonal control of rat liver regeneration. Gastroenterology 76: 1470–1482 (1979)

Logothetopoulos, L., B.B. Sharma, J.M. Salter, C.H. Best: Glucagon and metaglucagon diabetes in rabbits. Diabetes 9: 278–285 (1960)

Munro, H.N., A. Fleck: The determination of nucleic acids. Meth. Biochem. Anal. 14: 113–178 (1966)

Petersen, K.F., B.A. Hansen, H. Vilstrup: Time dependent stimulatory effect of glucagon on the capacity of urea synthesis in rats. Hormone and Metabolic Research 19: 53–56 (1987)

Pilsgaard, H., H.E. Poulsen: A one-sample method for antipyrine clearance determination in rats. Pharmacology 29: 110–116 (1984)

Poulsen, H.: One sample antipyrine clearance after 90% partial hepatectomy in the rat. Liver 5: 200–204 (1985)

Requests for reprints should be addressed to: Kitt Falk Petersen, M.D., Department of Medicine A-2152, Rigshospitalet, 9 Blegdamsvej, DK-2100 Copenhagen (Denmark)