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Effect of Starvation Upon Hepatic Insulin Metabolism in the Rabbit

C. López-Quijada and E. Blázquez

Instituto Gregorio Marañón C.S.I.C. Departamento de Metabolismo
Velázquez, 144, Madrid - 6 (Spain)

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Plasma insulin levels and blood glucose in both the portal vein and the posterior vena cava in rabbits after total starvation have been studied. The mean plasma insulin of control group changes only from 34 μu/ml in the portal vein to 23 μu/ml in the posterior vena cava. Proportionally, during the fasting state a much smaller amount of insulin traverses the liver into the peripheral circulation from 21 μu/ml to 8 μu/ml. Glucose concentration after total starvation remains very stable (76 mg/100 ml).

Hepatic metabolism is drastically altered during starvation (7) and there are complex pathways which permit a finely regulated metabolic system.

Previous experimental evidence has suggested that insulin is the primary signal responsible for fuel control during starvation (3). After prolonged festing rat (9) as wel as man (1) shows a marked intolerance to a load of glucose. This could not be due to depletion of stores of pancreatic insulin as it has been shown (11) that fasting causes a marked reduction in the response of the islets to glucose long before there is any decrease in the insulin content of the pancreas. It has beem reported by BEST *et al.* (2) that after 7 days of starvation the pancreas of the rats contains little more than half its normal complement of the hormone.

This work was therefore planned to obtain information concerning the hepatic function and hormonal response to fasting in the hope that such information would provide us at least a partial insight into some of these problems.

To asses the part played by the insulin-secretory system in the release of insulin and the maintenance of blood glucose versus plasma insulin concentration, insulin secretion by rabbit's pancreas was studied after a fasting period of 5 days.

Materials and Methods

IMMUNOASSAY. The determination of insulin was carried out by method «C» of HALES and RANDLE (8). The bovine insulin used for standard in the immunological test was obtained from Eli Lilly Co. Indianapolis. Iodinated insulin-^{125}I (Radiochemical Center) was prepared from specially purified crystalline ox insulin (Borrough and Co., potency 24.3 U.I./mg) by iodination with iodine monochloride. Insulin-binding reagent was obtained from

Wellcome Laboratories. Insulin concentration expressed as microunits or micrograms is given as bovine equivalent since bovine insulin has always been used as the standard for different samples. The validity of the procedure has been established by the parallelism of the dilution curves of rabbit and bovine insulin.

Rabbits of about 2 kg body weight separated into groups were studied. 15 rabbits for group I (centrol) were deprived of food for 18 hours and other 15 rabbits of group II (starvation) were deprived of food for 5 days. During the time course of fasting the animals had free access to water.

All animals were anaesthetized with ether and blood was removed from either the portal vein (p.v.) or the posterior vena cava (p.v.c.). Small amounts of heparin were added to provent coagulation and plasma was separated by centrifugation. Specimens of blood were kept at room temperature no more than one hour. Blood sugar was determined by the SOMOGYI-NELSON procedure (13).

The entire pancreas of five rabbits of each group was removed for insulin extraction. Insulin from rabbit pancreas was extracted and partially purified according to COORE and RANDLE (5). The extrac was then diluted in 0.04 M phosphate buffer *p*H 7.4 to the required final concentration.

Results

EFEECT OF STARVATION ON BLOOD GLUCOSE AND PLASMA INSULIN CONCENTRATION. Plasma insulin concentration versus blood glucose has been studied in both the portal vein (p.v.) and the posterior vena cava (p.v.c.) in rabbits after total starvation.

Blood glucose and plasma insulin before starvation (control samples) are values obtained after a routine overnight fast. The mean plasma insulin in the p.v. changes only from 34 ± 7.4 to 21 ± 5.6, $P = 0.01$

Table I. *Effect of starvation on blood glucose and plasma insulin in rabbits.* Rabbits were divided into two equal groups. Values given as group A were obtained after a routine overnight fast. Values given as group B were obtained after five days fasting. Insulin values are given as equivalents of bovine insulin (potency 25 international units/mg). Figures given are in all cases mean values of ten experiments.

GROUP	PORTAL VEIN		POSTERIOR VENA CAVA	
	Plasma-Insulin (μu/ml)	Blood glucose (mg/100 ml)	Plasma-Insulin (μu/ml)	Blood glucose (mg/100 ml)
Group A. Before starvation	34±7.4	118±19.7	23±4.4	107±17.9
Group B. After starvation	21±5.6	76± 8.9	8±4.2	76±13.6
P * between A and B	=0.01	=0.01	< 0.001	=0.01

* «Student's test».

Table II. *Relationship between plasma insulin concentration in the portal vein (p.v.) and the posterior vena cava (p.v.c.) before and after starvation.*

Values given as before starvation, were obtained after a routine overnight fast. Values given as after starvation were obtained after five days fastig. Insulin values are given as equivalents of bovine insulin (potency 25 international units/mg). Figures given are in all cases mean values of ten experiments.

GROUP	Before starvation	After starvation
Plasma-insulin (μu/ml) (p.v.)	34±7.4	21±5.6
Plasma-insulin (μu/ml) (p.v.c.)	23±4.4	8±4.2
Significance	p *=0.01	p < 0.001
Insulin disapearance	33 %	62 %

* «Student's test».

while in the p.v.c. the values decreased much more pronounced (from 23 ± 4.4 to 8 ± 4.2, $P < 0.001$) (Table I).

On the other hand, after total starvation the relationship between insulin concentration in the portal vein (mean 21 ± 5.6) and the posterior vena cava (mean 8 ± 42) was very significant ($P < 0.001$) (Table II). Glucose concentration after total starvation remained very stable and the decrease is in very good agreemente with the levels of immunoreactive insulin in the posterior vena cava.

INSULIN CONTENT OF THE PANCREAS. When insulin content was compoared to the weigh of extracted tissue, it was found that the total amount of insulin which could be extracted from pancreas of normal rabbits (36 μg) was unaffected by fasting for five days (Table III). The total weight of the pancreas changes only slighly.

Discussion

The above findings provide some basis for understanding of the insulin metabolism during fasting. In order to know the influence of the liver on insulin metabolism during the fasting state, the levels of circulating insulin have been measured in both the portal and the posterior vena cava in rabbits after five days of complete starvation. In the control group, the insulin concentration changes only from 34 μu/ml in the portal vein to 23 μu/ml in the posterior vena cava. Proportionally, during the fasting state, a much smaller amount of insulin traverses the liver (from 21 μu/ml in the portal vein to 8 μu/ml in the posterior vena cava) into the peripheral circulation where its function almost certainly does not to affect the entry of glucose into cell tissues which use little glucose in fasting state.

These results suggest that the liver plays an importat role in control of the regulation of insulin levels, in order to maintain constancy of blood glucose during the fasting state. This possibility is in agreement with the suggestion that during starvation most of the insulin released by the pancreas is cleared from the portal circulation by the liver to modulate gluconeogenesis (6). On the other hand excessive insulin destruction induced by starvation reduces the effectiveness of insulin. MIRSKY and PERISUTTI (12) have demonstrated the presence of insulinase activity in rat liver slices rises during starvation. It has been speculated that this capacity of the liver to destroy the hormone is a regulatory device to protec the body against hypoglicemia.

The stimulus to insulin release in the fasting state is not known, but basal insulin release may occur autonomously (4). It has been suggested that the effect of total starvation upon the function of the beta cells of islets of Langerhans my be profound (14). It has been reported that after prolonged fasting there is a depletion of the store of pancreatic insulin in rats (2). Nevertheless our finding show that in rabbits assayable insulin is present in great amounts in dark as well as in pale

Table III. *Effect of total starvation on extractable pancreas insulin in rabbits.*

Rabbits were divided into equal groups. Values gives as group A were obtained after routine overnight fast. Values given as group B were obtained after five days fasting. Insulin values are as equivalents of bovine insulin (potency 25 international units/m). Figures given are in all cases mean values of ten experiments.

GROUP	Pancreas-insulin μg/100 mg tissue	PANCREAS	
		Total weight g	Total insulin μg
Group A. Before starvation	3.56 ± 0.15	0.99 ± 0.25	36 ± 8.1
Group B. After starvation	3.68 ± 0.34	0.93 ± 0.96	34 ± 8.4
P * between A and B	> 0.4	> 0.9	> 0.5

* «Student's test».

beta cell granules and possibly in cytoplasmic structures other that secretory granules (10).

Immunossay figures for insulin in pancreas from fasting rabbit have not been reported so far. Our findings confirm taht there is a good correlation between the insulin assay figures of fasting rabbit pancreas on one hand and light and electron micoscopic beta cell granulation on the other if pale granules are assumed to contain immunologically active insulin (10).

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