

EFFECTS OF A PORTACAVAL SHUNT ON THE ENTEROINSULAR AXIS IN RATS

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We investigated the effect of a portacaval shunt on the enteroinsular axis in rats, 8 weeks after surgery. Fasting plasma levels of insulin, glucagon, somatostatin and glucose, as well as pancreatic content of insulin, glucagon, and somatostatin were determined. In addition, determination of the fasting plasma level and concentration of GLP-1 in tissue extracts of ileum, as well as morphometric analysis of GLP-1-immunoreactive cells in different segments of the small intestine were included. Normal plasma levels of insulin and somatostatin, accompanied by hyperglucagonemia, and hypoglycemia were observed. Plasma level of GLP-1, and the number of GLP-1-immunoreactive cells in ileum were significantly reduced in rats with a portacaval shunt compared with control animals. However, concentrations of GLP-1 in tissue extracts of the ileum of portacaval shunted rats were unchanged. These results suggest that the abnormalities of the enteroinsular axis present in rats with a portacaval shunt were due to the functional deterioration of the liver.

Key words: enteroinsular axis, GLP-1, portacaval shunt, rat

INTRODUCTION

The incretin concept comprises the interaction of a hormonal gastrointestinal principle with glucose-induced insulin release from the pancreas. The components of the enteroinsular system, which are basic for the incretin effect, are endocrine transmission in combination with substrate stimulation of the pancreatic islets by glucose (Kofod, 1992). Even although the majority of hormones belonging to the secretin/glucagon family potentiate glucose-stimulated insulin release, only two of all the gastrointestinal hormones known today fulfill both requirements for being an incretin: gastric inhibitory polypeptide (GIP), (Tseng *et al.*, 1996), and glucagon-like peptide-1 (GLP-1) (Holst *et al.*, 1997). Both GIP and GLP-1 stimulate the secretion of insulin in pancreatic β - cells and are, therefore, likely to play important roles in the regulation of postprandial glucose homeostasis in man.

An incretin role for GLP-1 would imply that physiological increments of GLP-1 concentrations in plasma are accompanied by evidence of stimulated insulin secretion during the postprandial phase of physiological hyperglycemia (Kieffer and Habener, 1999, Doyle and Egan, 2001).

Glucagon-like peptide-1 (GLP-1) is one of the incretin hormones released from the gut and it stimulates insulin secretion, playing a very important role in the enteroinsular axis, as the primary hormonal regulator. GLP-1 stimulates insulin secretion during the postprandial phase of physiological hyperglycemia, suppresses glucagon secretion, stimulates (pro)-insulin biosynthesis and decreases the rate of gastric emptying and acid secretion. It has also been shown to have a pro-satiety effect. This representative humoral incretin is an excellent candidate for the treatment of patients with type 2 diabetes mellitus (Kieffer and Habener, 1999; Perfetti and Merkel, 2000; Doyle and Egan, 2001).

GLP-1, released into the portal circulation in response to ingestion of a meal, exerts an insulintropic action through binding to the truncated GLP-1 (tGLP-1) receptor known to exist in a single molecular form thus far. The hepatic vagal nerve is receptive to intraportal tGLP-1, through a mechanism mediated by a specific receptor to the hormone. The neural action of tGLP-1 involves a receptor mechanism distinct from that in the well-known humoral insulintropic action. tGLP-1 plays a role as a neuroincretin in the enteroinsular axis, in concert with its role as a humoral incretin (Nishizawa *et al.*, 2000).

Some endocrine consequences of a portocaval shunt (PCS) have been reported in the rat (Bani 1994), including abnormalities in glucose homeostasis and pancreatic content of hormones (Radosavljević *et al.*, 2001). Since GLP-1 is a major incretin and its secretion could be regulated by circulating glucose and insulin (Sugiyama *et al.*, 1996), the aim of this study was to investigate the entero-insular axis, particularly GLP-1 and hormones of the endocrine pancreas in an experimental model of chronic liver insufficiency (PCS performed in rats).

MATERIAL AND METHODS

Animals and experimental design. Two-month-old male Wistar rats, weighing approximately 230 g were maintained at room temperature in a 12-h dark, 12-h light cycle. They had free access to a conventional chow diet and tap water and were kept in separate cages. The study was approved by the institutional ethics committee for animal experiments. The animals were divided into two groups: control non-operated (C rats; $n=11$) and rats with a surgical portacaval shunt (PCS rats; $n=27$). On day 1 of the experiment the rats underwent end-to-side portacaval anastomosis according to Lee and Fisher (1961) as modified by Bismuth and Benhamon (1963). The surgery was performed under ether anesthesia. All the animals were killed by cervical dislocation eight weeks later. Twenty-four hours before sacrifice the feed was withdrawn, but the rats had access to tap water.

Analytical methods. Just prior to sacrifice blood samples were taken from the retroorbital venous plexus and serum/plasma was stored at -20°C until required. All the biochemical variables were measured in serum/plasma samples from individual rats. Basal plasma glucose was measured by the glucose oxidase method using a glucose analyser 2 (Beckman, Fullerton, CA). Upon sacrifice the liver and pancreas were removed from each animal and weighed. Some of the specimens from the juxtasplenic part of the pancreas and ileum was extracted as

follows. The tissues were weighed, placed in plastic tubes and immediately extracted for 10 min in 10 vol. of 0.5 M acetic acid in a water bath at 100°C. After cooling, the tubes were stored at -20°C until analysis.

Radioimmunoassay (RIA) methods. Just prior to being killed, blood samples were collected by intracardial puncture in heparinized tubes and centrifuged to separate the plasma. Serum was also obtained after coagulation. The basal serum levels of insulin and plasma glucagon were determined by radioimmunoassay (INEP-Diagnostics, Zemun and NovoBioLabs, Bagsvaerd, Denmark, respectively). Basal plasma somatostatin was measured using a commercial kit (Proenix, Pharmaceuticals, Inc. Affinity Research Products Ltd). Pancreatic content of insulin, glucagon, and somatostatin was measured using the same RIA kits as above. The concentration GLP-1 in plasma and tissue extract of small intestine was measured using the RIA kit (Proenix Pharmaceuticals, Inc. - Affinity Research Products Ltd.) (Human glucagon-like peptide 1 (7-37), Cat. no. RK-028-13).

Immunohistochemical studies. Total enteroendocrine cell, chromogranin-A-producing cells, as well as GLP-1-immunoreactive cells were counted in different segments of the small intestine of C and PCS animals using the PAP immunohistochemistry technique.

Statistical analysis. All values are expressed as mean \pm SD. Statistical analysis of data was made using the Mann Whitney nonparametric test and $p < 0.05$ was accepted as significant.

RESULTS

Body weights were not significantly different between the groups 8 weeks after the operation. Liver weight was significantly lower in PCS rats than in control rats ($p < 0.01$). The same was observed when liver weight was expressed as a percentage of body weight ($p < 0.01$). Pancreas weight was significantly greater in PCS than in control rats either expressed in absolute values ($p < 0.01$) or as a percentage of body weight ($p < 0.05$).

Table 1. Absolute and relative weights of liver and pancreas in C and PCS rats at the end point of the experiment

WEIGHTS	C (N=11)	PCS (N=27)
BODY WEIGHT (g)	234 \pm 12	236 \pm 34
LIVER WEIGHT (g)	6.9 \pm 1.3	5.1 \pm 1.2**
% of body weight	2.9 \pm 0.5	2.1 \pm 0.2**
PANCREATIC WEIGHT (g)	0.77 \pm 0.27	1.12 \pm 0.38**
% of body weight	0.33 \pm 0.11	0.49 \pm 0.17*

** $p < 0.01$, * $p < 0.05$

In rats with PCS basal plasma level of glucose was significantly lower compared with control animals (4.02 ± 1.08 vs. 5.19 ± 1.06 mmol/l, respectively, $p < 0.01$).

Basal serum level of insulin and plasma somatostatin, as well pancreatic content of somatostatin and insulin were not significantly different from controls. PCS rats showed a markedly increased plasma glucagon concentration compared with control rats ($p < 0.01$). Moreover, pancreatic glucagon content was increased ($p < 0.01$).

Table 2: Insulin, glucagon and somatostatin in serum/plasma and pancreatic tissue extracts of control and rats with portacaval shunt at the end point of the experiment

Animals	INSULIN		GLUCAGON		SOMATOSTATIN	
	Serum (mIU/l)	Pancreatic tissue content (mIU/g)	Plasma (pg/ml)	Pancreatic tissue content (pg/g)	Plasma (pmol/l)	Pancreatic tissue content (pmol/g)
C	20.6 ± 12	14.6 ± 6.9	196.5 ± 44	1256 ± 59.4	70.5 ± 10.9	400 ± 56
PCS	17.7 ± 8.0	12.2 ± 7.3	$555 \pm 9^{**}$	$2280 \pm 53.7^{**}$	73.2 ± 8.6	385 ± 62

$^{**}p < 0.01$

Morphometric analyses of total enteroendocrine cell counts, chromogranin-A-producing cells, as well as GLP-1-immunoreactive cells in different segments of the small intestine of C and PCS animals are presented in Table 3.

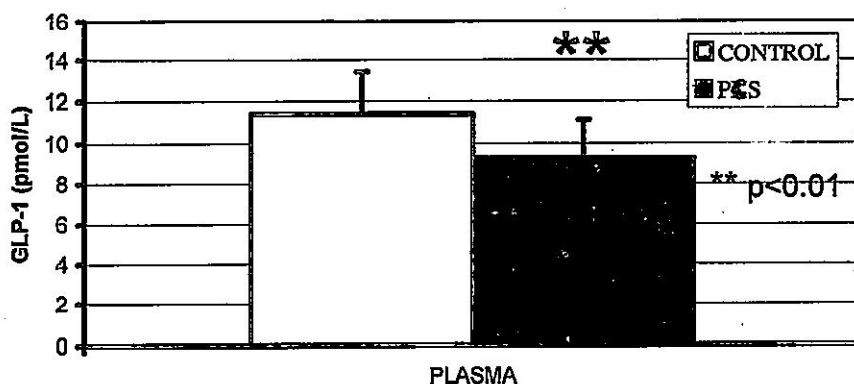


Figure 1. The mean basal concentration of GLP-1 in plasma of C and PCS rats at the end point of the experiment

The basal values of GLP-1 in plasma and tissue extract of ileum are shown in Figures 1 and 2. The mean concentration of GLP-1 in plasma was significantly reduced in rats with PCS, compared with C rats, (Figure 1), while the mean concentration of GLP-1 in tissue extracts of ileum was unchanged (Figure 2).

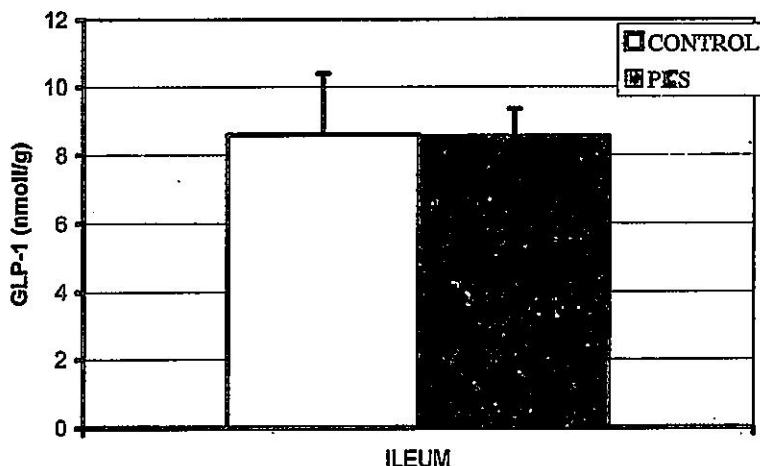


Figure 2. The values of GLP-1 in tissue extracts of ileum in C and PCS rats at the end point of the experiment

An immunocytochemical study of GLP-1-immunoreactive cells in different segments of the small intestine showed a similar distribution of these cells in C and PCS rats. However, the number of GLP-1-immunoreactive cells in the ileum of rats with PCS was reduced, compared with control animals.

Table 3. Morphometric analysis of total endocrine and GLP-1-immunoreactive cells in different segments of the small intestine of C and PCS at the end point of the experiment

Number of endocrine cells / 1000 epithelial cells of the mucosa.			
	Duodenum	Jejunum	Ileum
HgA- immunoreactive			
C (n=11)	17,6 ± 2,6	22,1 ± 4,7	19,7 ± 5,6
PCS (n=25)	18,1 ± 3,4	22,7 ± 4,0	20,0 ± 4,3
GLP-1-immunoreactive			
C (n=10)	4,3 ± 0,5	4,6 ± 0,3	5,7 ± 0,7
PCS (n=24)	4,5 ± 0,6	4,5 ± 0,2	4,6 ± 0,2*

HgA-chromogranin A; * $p < 0,05$

DISCUSSION

The end-to-side porta-caval shunt (PCS) in rats provides an experimental model of chronic liver insufficiency such as occurs in cirrhosis and porto-systemic shunt. It produces marked alterations in the metabolic, nutritive and hormonal states (Kravetz *et al.*, 1987; Bani *et al.*, 1994; Milošević *et al.*, 1996; Lin *et al.*, 1996; Radosavljević *et al.*, 1997). Complete diversion of the portal blood

flow from the liver leads to impairment of its metabolic functions and atrophy of hepatocytes.

GLP-1 is one of the incretin hormones released from the gut. It stimulates insulin secretion and plays an important role in the enteroinsular axis.

In the current study, basal plasma values of GLP-1 were significantly reduced in rats with PCS, compared with C rats, while concentrations of GLP-1 in tissue extracts of ileum were unchanged. A immunocytochemical study of GLP-1-immunoreactive cells in different segments of the small intestine showed similar distribution of these cells in C and PCS rats. However, the number of GLP-1-immunoreactive cells in the ileum of rats with PCS was reduced, compared with control animals. Other authors have shown that in cirrhosis there is impaired release of GLP-1 or resistance to GLP-1 action (Kruszynska *et al.*, 1995).

The results of our study also showed that, 8 weeks after PCS, there are normal circulating levels of insulin and somatostatin, but hyperglucagonemia and hypoglycemia. Reduction of hepatic glycogen content in PCS rats was recorded in a previous study (Šikić *et al.*, 2001).

In the present study, basal plasma level of glucagon and pancreatic content of this hormone was increased in PCS rats compared with control rats. This confirms earlier results indicating hyperglucagonemia but a normal secretion pattern of A-cells in rats with PCS (Radosavljević *et al.*, 2000; 2001). Thus, hyperglucagonemia was probably not caused by hypersecretion of A-cells, but by reduction of hepatic extraction of glucagon. Other studies also showed that hyperglucagonemia and hypoglycemia were accompanied by a reduction of hepatic glycogen content in rats with PCS (Bani *et al.*, 1994). Decreased hepatic catabolism resulting from progressively impaired hepatic function may play a role in the development of hyperglucagonemia in patients with cirrhosis and portal hypertension (Lin *et al.*, 1996). However, other results suggest that pancreatic hypersecretion of glucagon may also contribute to increased plasma glucagon levels in patients with cirrhosis and portocaval anastomosis (Dupre *et al.*, 1991).

Our findings indicate that in rats with PCS, mean basal plasma somatostatin concentration and pancreatic somatostatin content were not changed. Normal immunoreactivity and ultrastructural patterns of D-cells in rats with PCS may explain these findings (Bani *et al.* 1994; Radosavljević *et al.*, 2001). In contrast to our results other authors observed that elevated levels of circulatory somatostatin in liver cirrhosis are associated with hyperglucagonaemia and impaired insulin release, while the high plasma somatostatin results from hypersecretion of the D-cells (Verrillo *et al.* 1986, Barreca *et al.* 1991).

One of the most persistent consequences of the end-to-side PCS in rats was the reduction of body weight gain during the first 2-4 postoperative weeks in spite of ad libitum feeding (Milošević *et al.* 1996). After that period, body weight gain in rats with PCS coincided with the increase in animals subjected to the sham procedure as well as in controls, 8 weeks postoperative. All metabolic changes developing in this period were PCS-specific and independent of feed intake. This experimental model of chronic hepatic insufficiency also causes metabolic disorders, such as the hyperammonemia noted in a previous study (Radosavljević *et al.*, 1998). Hyperammonemia is most probably the consequence of complete diversion of portal blood rich in ammonia into the systemic circulation and decreased liver capacity for ammonia uptake. Hyperammonemia has a toxic effect on the liver, inducing increased permeability of hepatocyte membranes with significantly increased concentrations of serum transaminases (GOT and GPT).

There was a significant positive correlation between ammonia and transaminase concentrations (Radosavljević *et al.*, 1998). However unchanged concentrations of blood urea (Radosavljević *et al.*, 1997), indicated unimpairment of one synthetic function of hepatocytes in PCS rats.

However, a porta-caval shunt does cause liver atrophy, manifested by a significant reduction of absolute liver weight and as a percentage of body weight. There are different opinions about the development of liver atrophy in PCS. It is considered to be a consequence of liver blood flow reduction (Assal *et al.* 1971, Kravetz *et al.* 1987), or a result of deprivation of hepatotrophic substances normally present in portal blood.

PCS causes pancreatic hypertrophy (Kravetz *et al.*, 1987; Nylander *et al.*, 1993) as confirmed in our study which suggests that PCS may raise the sensitivity of CCK-A receptors in the pancreas to CCK through increased concentrations of intestinal factors in the circulation (Nylander *et al.*, 1993).

The present results indicate that the functional deterioration of the liver, which follows the diversion of the portal blood into the systemic circulation, may cause abnormalities of the entero-insular axis, particularly GLP-1 leading to changes in hormonal status of the endocrine pancreas and metabolism of carbohydrate.

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EFEKTI PORTO-KAVNOG ŠANTA NA ENTEROINSULARNU OSOVINU U PACOVA

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SADRŽAJ

U ovom radu je ispitivan efekat porto-kavnog šanta na enteroinsularnu osovinu kod pacova, 8 nedelja nakon operacije. Određivane su bazalne vrednosti insulina, glukagona, somatostatina i glukoze u plazmi, kao i koncentracije insulina, glukagona i somatostatina u ekstraktu tkiva pankreasa. Merene su vrednosti GLP-1 u plazmi i ekstraktu ileuma, a izvršena je i morfometrijska analiza GLP-1-imunoreaktivnih ćelija u različitim segmentima tankog creva. Bazalne vrednosti insulina i somatostatina u plazmi bile su normalne, a registrovana je hiperglukagonemija i hipoglikemija kod pacova sa porto-kavnim šantom. Vrednosti GLP-1 u plazmi su bile značajno redukovane kod operisanih životinja u odnosu na kontrolne, dok su tkivne koncentracije GLP-1 u ileumu bile nepromenjene. Međutim, nađeno je smanjenje GLP-1-produkujućih ćelija u ileumu kod pacova sa PKS. Rezultati ove studije pokazuju poremećaje enteroinsularne osovine u pacova sa porto-kavnim šantom koji su nastupili kao posledica funkcionalnog oštećenja jetre.