

# Portal glucose infusion increases hepatic glycogen deposition in conscious unrestrained rats

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**Cardin, Sylvain, Maya Emshwiler, Patricia A. Jackson, Wanda L. Snead, Jon Hastings, Dale S. Edgerton, and Alan D. Cherrington.** Portal glucose infusion increases hepatic glycogen deposition in conscious unrestrained rats. *J. Appl. Physiol.* 87(4): 1470–1475, 1999.—It has been demonstrated in the conscious dog that portal glucose infusion creates a signal that increases net hepatic glucose uptake and hepatic glycogen deposition. Experiments leading to an understanding of the mechanism by which this change occurs will be facilitated if this finding can be reproduced in the rat. Rats weighing 275–300 g were implanted with four indwelling catheters (one in the portal vein, one in the left carotid artery, and two in the right jugular vein) that were externalized between the scapulae. The rats were studied in a conscious, unrestrained condition 7 days after surgery, following a 24-h fast. Each experiment consisted of a 30- to 60-min equilibration, a 30-min baseline, and a 120-min test period. In the test period, a pancreatic clamp was performed by using somatostatin, insulin, and glucagon. Glucose was given simultaneously either through the jugular vein to clamp the arterial blood level at 220 mg/dl (Pe low group) or at 250 mg/dl (Pe high group), or via the hepatic portal vein (Po group; 6 mg·kg<sup>-1</sup>·min<sup>-1</sup>) and the jugular vein to clamp the arterial blood glucose level to 220 mg/dl. In the test period, the arterial plasma glucagon and insulin levels were not significantly different in the three groups (36 ± 2, 33 ± 2, and 30 ± 2 pg/ml and 1.34 ± 0.08, 1.37 ± 0.18, and 1.66 ± 0.11 ng/ml in Po, Pe low, and Pe high groups, respectively). The arterial blood glucose levels during the test period were 224 ± 4 mg/dl for Po, 220 ± 3 for Pe low, and 255 ± 2 for Pe high group. The liver glycogen content (μmol glucose/g liver) in the two Pe groups was not statistically different (51 ± 7 and 65 ± 8, respectively), whereas the glycogen level in the Po group was significantly greater (93 ± 9, *P* < 0.05). Because portal glucose delivery also augments hepatic glycogen deposition in the rat, as it does in the dogs, mechanistic studies relating to its function can now be undertaken in this species.

liver; somatostatin; insulin; glucagon; portal signal

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THE LIVER IS ONE OF THE KEY ORGANS in glucose homeostasis. Whereas a great deal is known about the liver as a producer of glucose, much less is known about its role in glucose disposal. It remains unclear exactly how hepatic glucose uptake is regulated after oral glucose consumption, when the blood glucose and insulin levels rise and the glucagon level falls. Based on work carried out in humans (11, 12) and in dogs (14, 18), it is clear that neither hyperinsulinemia nor hyperglycemia, when

brought about independently, cause much net glucose uptake by the liver. Even when hyperglycemia (induced by peripheral glucose administration) and hyperinsulinemia are combined, net hepatic glucose uptake is still lower than the rates seen after oral glucose consumption (11, 26, 30). Thus the liver seems to take up glucose maximally only when the glucose is ingested or when it is delivered via the hepatic portal vein, as it occurs during absorption (4, 5, 16).

Adkins et al. (1, 2) have clearly demonstrated in the conscious dog (using the pancreatic clamp to maintain the hormonal milieu) that, for the same hepatic glucose load, the liver takes up significantly more glucose if a portion of the glucose enters the hepatic portal vein. These studies have made clear that a portal signal plays an important role in the uptake of glucose by the liver. This portal signal augments net hepatic glucose uptake regardless of the glucose load to the liver and in the presence of both basal and elevated insulin levels (22, 23). It has also been demonstrated that the net hepatic glucose uptake is directly proportional to the negative arterial-portal glucose gradient, which is an index of the rate of intraportal glucose delivery (22, 23, 28).

The central nervous system seems to be involved in this phenomenon, since denervation of the liver or intraportal infusion of adrenergic blockers and acetylcholine (known to mimic changes in neural input to the liver) reduced or increased, respectively, net hepatic glucose uptake in response to portal glucose delivery (7, 20). Because postprandial hyperglycemia is a major problem in patients with diabetes, and a normal postprandial increase in net hepatic glucose uptake is absent in diabetic individuals (34), a better understanding of the regulation of net hepatic glucose uptake could eventually lead to improved treatment of the disease.

Although the dog is a good model for the experiments listed above, variability in brain morphology, combined with poor brain stereotaxic information in this species, prohibits its use for studies related to the role of the central nervous system in metabolic function. Because we were interested in understanding the involvement of the central nervous system in the regulation of net hepatic glucose uptake, one of our objectives was to develop a model of portal signaling in a small-animal model. Because of the similarity in brain morphology from animal to animal, and the availability of detailed brain stereotaxic information for the rat, we chose this species for study.

Our aim, therefore, was to determine whether the portal signal, effective in the dog and human, is also effective in the rat. Since it has been demonstrated in

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the dog that the primary fate of the glucose taken up by the liver in response to the portal signal is deposition as glycogen (20, 27), we investigated the effects of portal glucose infusion on glycogen deposition in unrestrained, conscious pancreatic-clamped rats.

## MATERIALS AND METHODS

**Animal care.** Male Sprague-Dawley strain rats (Harlan, Indianapolis, IN) weighing 275–300 g were housed as a group and maintained on rat Purina chow and water ad libitum. They were housed under conditions of controlled temperature ( $21 \pm 2^\circ\text{C}$ ) and light (7:00 AM to 7:00 PM). Twenty-four hours before the study, the animals were deprived of food to deplete liver glycogen but were allowed free access to water. Only those animals that had recovered their presurgery weight and exhibited normal activity were used. To avoid stress due to manipulation, rats were handled each day in the experimental environment. Animal housing met the standards of the American Association for the Accreditation of Laboratory Animal Care, and the protocol was approved by the Vanderbilt University Medical School Animal Care Committee.

**Surgical procedures.** Eight days before the experiment, rats underwent a carotid artery, a jugular vein, and a hepatic portal vein cannulation under pentobarbital sodium anesthesia (50 mg/kg ip, Abbott Laboratories, North Chicago, IL). Two polyethylene catheters (PE 10, 0.28 mm ID, 0.61 mm OD; Intramedic, Clay Adams, Sparks, MD) were introduced into the right jugular vein after blunt dissection. The left carotid artery was visualized after the separation of the sternomastoides and the sternohyoideus muscles, and a polyethylene catheter (PE 50, 0.58 mm ID, 0.965 mm OD; Intramedic, Clay Adams) was implanted. The hepatic portal catheter was inserted according to the technique described by Cardin et al. (6) with some modification. Briefly, a 2- to 3-cm laparotomy incision was performed, the cecum was lifted out of the peritoneum, two mesenteric vessels draining to the ileocolic vein were located, and the area at the junction of these two vessels was cleared of fat. A small incision was made, and a catheter (Silastic tubing, 0.51 mm ID, 0.91 mm OD, Dow Corning no. 508–002) was threaded toward the portal vein. All the catheters were tunneled under the skin. Their conically shaped anchor (a silicone medical adhesive type A), in which all the catheters were glued together, was fixed between the shoulder blades. To avoid clot formation, a heparinized (200 U/ml) saline solution was introduced into the catheters just before they were sealed.

**Experimental design.** On the day of experimentation, the rat was placed in a transparent experimental cage in the procedure room. After 30 min, all catheters were opened, cleared of heparinized saline, and connected to syringes on Harvard infusion pumps (Harvard Apparatus, South Natick, MA). Once the rats were calm and accustomed to the experimental setup (between 30 and 60 min later), the first blood sample was taken, and the time was identified as “–30 min.” Thereafter, blood samples were taken at 30-min intervals until 120 min. At *time 0*, somatostatin was infused through the jugular vein ( $1.3 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , Bachem, Torrance, CA) to gain control of the endocrine pancreas. Glucagon ( $0.8 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , Eli Lilly, Indianapolis, IN) and porcine regular insulin ( $2 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , Eli Lilly) were infused intraportally for the remainder of the study. Glucose (50% dextrose, Abbott Laboratories, North Chicago, IL) was infused through the jugular catheter to maintain the arterial blood glucose level at 220 mg/dl (Pe low group) or 250 mg/dl (Pe high group). In the third group, the portal signal was induced by combining an infusion of  $6 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  of glucose into the hepatic

portal vein, with a variable glucose infusion through the jugular vein, to clamp the arterial blood glucose level at 220 mg/dl (Po group). Our goal was to achieve steady state by 30 min. Blood glucose was sampled every 5–10 min to maintain the clamp. To avoid complications resulting from blood sampling, the red blood cells of the rat were suspended in saline and reinjected as soon as possible (<20% of the rat's total blood volume was removed). After an experiment, the animal was injected intrajugularly with pentobarbital sodium (25 mg/kg), the abdomen was quickly opened, and a small piece of the caudal lobe of the liver was freeze-clamped with aluminum tongs precooled in liquid nitrogen, after which the animal was killed by pneumothorax. A group of six control animals was treated in an identical fashion (i.e., diet, housing, catheter insertion, etc.), except that in this group no solutions were administered, and liver tissue was obtained for assessment of the basal glycogen concentration.

**Analytical procedures.** Blood glucose levels were assayed by using an enzymatic photometric method with an hemocue glucose analyzer (HemoCue, Mission Viejo, CA). Plasma insulin and glucagon were measured by using a species-specific double-antibody radioimmunoassay, as described previously (21), with interassay coefficients of variability of 11 and 8%, respectively. The antibodies and  $^{125}\text{I}$ -labeled tracer were purchased from Linco Research (St. Louis, MO). Plasma samples for glucagon determination were preserved by addition of aprotinin (Trasylol, Bayer, Kankakee, IL) at collection. Hepatic glycogen levels were determined by using the amyloglucosidase method described by Keppler and Decker (17).

**Statistical analysis.** Data are expressed as means  $\pm$  SE with 8–10 animals/group. Statistical comparisons between groups were made by using analysis of variance. Post hoc analysis was performed by using a Tukey-Kramer multiple-comparisons test. Statistical significance was accepted at  $P < 0.05$ .

## RESULTS

The average glucose levels in the basal period were similar in the three groups (Po,  $95 \pm 1 \text{ mg/dl}$ ; Pe low,  $95 \pm 1 \text{ mg/dl}$ ; Pe high,  $94 \pm 1 \text{ mg/dl}$ ). The average arterial blood glucose levels for the experimental period were  $224 \pm 4$ ,  $220 \pm 3$ , and  $255 \pm 2 \text{ mg/dl}$ , respectively, in Po, Pe low, and Pe high groups (Fig. 1). There was no statistical difference between the plasma glucagon levels (Po,  $36 \pm 2 \text{ pg/ml}$ ; Pe low,  $33 \pm 2 \text{ pg/ml}$ ; Pe high,  $30 \pm 2 \text{ pg/ml}$ ; Fig. 2, *top*). Similarly, the average arterial plasma insulin levels during the experimental period were not significantly different (Fig. 2, *bottom*) among the groups (Po,  $1.34 \pm 0.08 \text{ ng/ml}$ ; Pe low,  $1.37 \pm 0.18 \text{ ng/ml}$ ; Pe high,  $1.66 \pm 0.11 \text{ ng/ml}$ ). The hepatic glycogen content for the control group was  $6 \pm 1 \mu\text{mol glucose/g liver}$ . In the Po group, the hepatic glycogen content ( $93 \pm 9 \mu\text{mol glucose/g liver}$ ) was significantly greater ( $P < 0.05$ ) than in the Pe low ( $51 \pm 7 \mu\text{mol glucose/g liver}$ ) and the Pe high groups ( $65 \pm 8 \mu\text{mol glucose/g liver}$ ; Fig. 3, *top*). The ratio of the change in hepatic glycogen content ( $\mu\text{mol glucose/g liver}$ ; the difference between the glycogen content in the experimental groups and the control group) to the change in insulin level (ng/ml) was significantly greater ( $P < 0.05$ ) in the Po group ( $84 \pm 9$ ) than in the Pe low ( $48 \pm 9$ ) or the Pe high ( $42 \pm 8$ ) groups, which, in turn, were not statistically different from each other (Fig. 3, *bottom*).

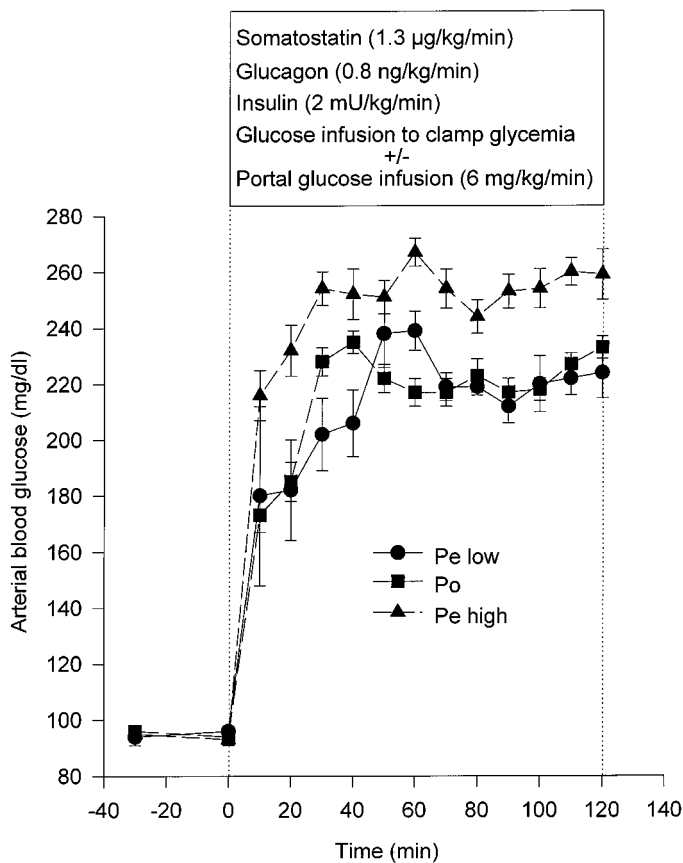


Fig. 1. Arterial blood glucose during the basal and test periods in pancreatic-clamped 24-h-fasted rats in which glycemia was maintained at 220 mg/dl (Po group) by jugular and portal (6  $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) vein glucose infusion and at 220 mg/dl (Pe low group) and 250 mg/dl (Pe high group) by jugular glucose infusion. Values are means  $\pm$  SE;  $n = 8-10$  rats/group.

The amount of glucose infused to maintain the blood glucose levels between the 30th and 120th minute were similar in the Po group ( $1,680 \pm 76$  mg/kg; portal + peripheral infusion) and the Pe low group ( $1,399 \pm 162$  mg/kg), but was significantly greater ( $P < 0.05$ ) in the Pe high group ( $2,408 \pm 235$  mg/kg).

## DISCUSSION

There are three major regulators of hepatic glycogen deposition: the insulin level, the hepatic glucose load, and the portal signal. The present experiments were designed to isolate the effect of the portal signal on hepatic glycogen deposition. The results of the present study indicate that the portal signal does increase hepatic glycogen deposition in conscious, unrestrained rats. This is demonstrated by the fact that the Po group, which received an infusion of glucose into the hepatic portal vein (creating an arterial-portal glucose gradient of approximately  $-12$  to  $-14$  mg/dl), deposited significantly more hepatic glycogen than did the other groups.

Glucose infusion at  $4 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  in the dog produces an arterial-portal glucose gradient of approximately  $-14$  mg/dl, and this is known to produce a maximal effect on net hepatic glucose uptake (27).

Because liver blood flow in the rat is  $\sim 50\%$  higher than in the dog, glucose was infused into the hepatic portal vein in the present study at a rate 50% higher than in the dog ( $6 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ). Assuming portal blood flow of  $34 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ , this would have resulted in an arterial-portal glucose gradient of  $-12$  to  $-14$  mg/dl (which is similar to the gradient usually used in studies in the dog). Although we cannot be certain that the dose response between the arterial-portal glucose gradient and the net hepatic glucose uptake is similar in the rat and the dog, it seems like a reasonable assumption.

The arterial plasma insulin and glucagon levels were reasonably similar in all three groups. The plasma

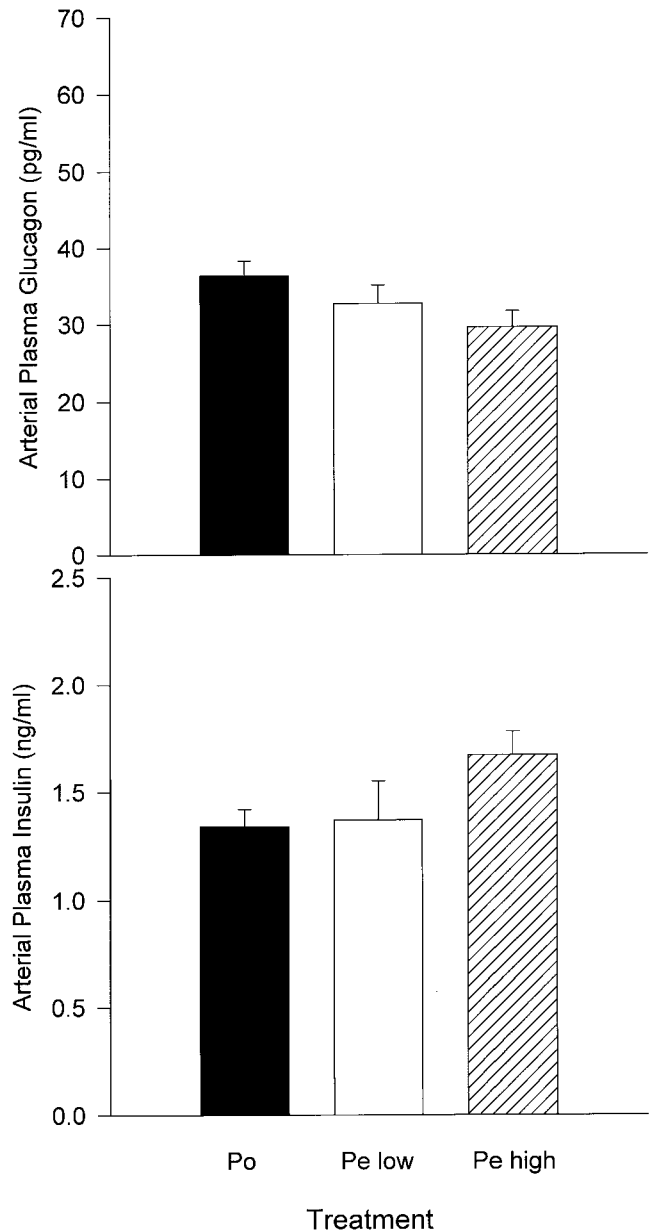


Fig. 2. Arterial plasma glucagon (top) and insulin (bottom) levels during test period in pancreatic-clamped 24-h-fasted rats in which glycemia was maintained at 220 mg/dl (Po) by jugular and portal (6  $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) vein glucose infusion and at 220 mg/dl (Pe low) and 250 mg/dl (Pe high) by jugular glucose infusion. Values are means  $\pm$  SE;  $n = 8-10$  rats/group.

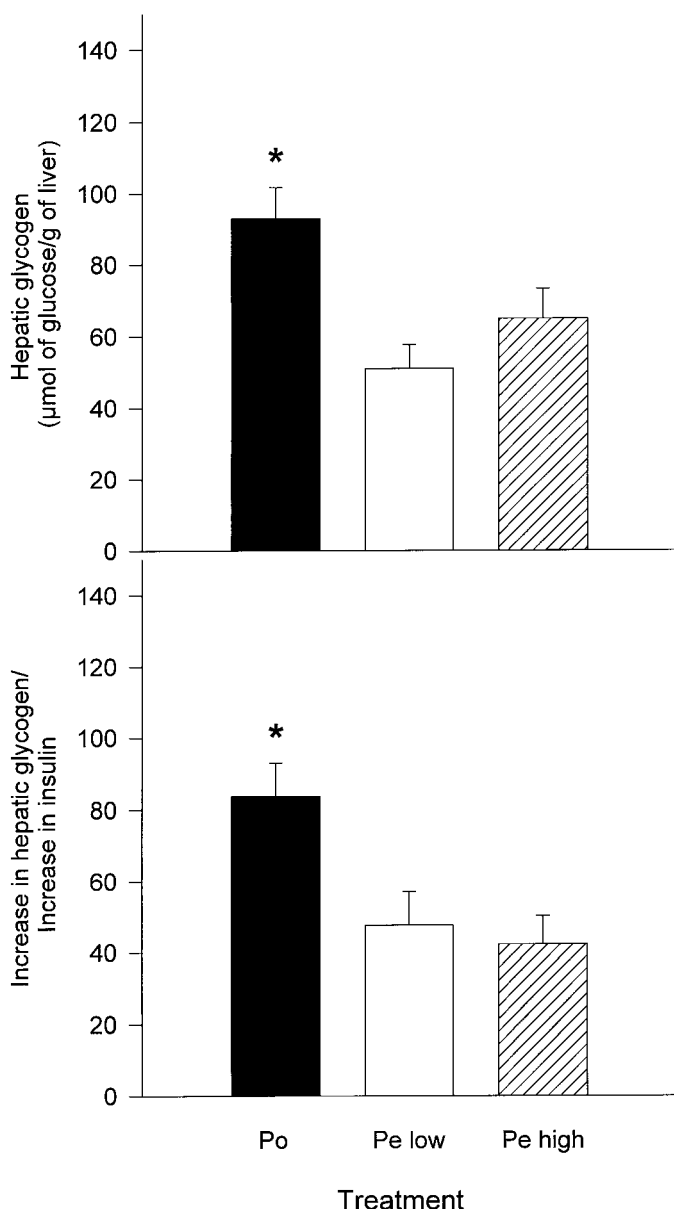


Fig. 3. Hepatic glycogen content (*top*) and change in hepatic glycogen content divided by increase in arterial plasma insulin levels (*bottom*) over test period in pancreatic-clamped 24-h-fasted rats in which glycemia was maintained at 220 mg/dl (Po) by jugular and portal (6 mg·kg<sup>-1</sup>·min<sup>-1</sup>) vein glucose infusion and at 220 mg/dl (Pe low) and 250 mg/dl (Pe high) by jugular glucose infusion. Values are means ± SE; *n* = 8–10 rats/group. \*Significantly different from 2 other groups (*P* < 0.05).

insulin level in the Pe high group was slightly (~20%) higher than in the Po group, but, if anything, this would minimize the increment in glycogen deposition caused by portal glucose delivery. In addition, when glycogen deposition was expressed relative to the rise in insulin, an effect of the portal signal was even more apparent. Indeed, this ratio was enhanced almost twofold in the presence of the portal signal.

Another factor that is important to hepatic glycogen deposition is the hepatic glucose load. The hepatic glucose load can be estimated by knowing the arterial glucose level, the portal glucose infusion rate (6

mg·kg<sup>-1</sup>·min<sup>-1</sup>), and the total hepatic blood flow. We assumed that total hepatic blood flow was ~50 ml·kg<sup>-1</sup>·min<sup>-1</sup>, of which 72% was derived from the portal vein (8). Using these parameters, we estimated that the glucose loads to the liver in the experimental period were 117 ± 4, 108 ± 3, and 125 ± 2 mg·kg<sup>-1</sup>·min<sup>-1</sup> in the Po, Pe low, and Pe high groups, respectively. It is obvious, therefore, that differences in glucose level in the sinusoidal circulation and the glucose load to the liver cannot explain the difference observed in hepatic glycogen deposition.

Because different regions of the brain have been demonstrated to sense the glucose level (3, 13, 19, 29) and because activation of different areas in the brain can, in turn, modify the enzymatic activity of the liver (31–33), we also controlled for the glucose level in the brain. It was identical in the Pe low and Po groups (220 vs. 224 mg/dl, respectively) and slightly higher in the Pe high group (254 mg/dl). The two Pe groups, despite having different arterial glucose levels, still had the same amount of hepatic glycogen deposition and the same Δinsulin/Δhepatic glycogen (where Δ means change), suggesting that the brain glucose level was not a factor in the response.

Therefore, the only variable that could explain greater hepatic glycogen deposition or greater Δinsulin/Δhepatic glycogen is portal glucose delivery (i.e., the negative arterial-portal gradient). It is postulated that the portal glucose level is sensed in the portal vein by glucose sensors that inform the brain, probably by the vagus nerve, of the nutritional state. It has been proposed, during hypoglycemia, for example, that these glucose sensors detect the low blood glucose level and stimulate catecholamine release (15). After portal glucose delivery, on the other hand, these sensors detect the increase in the portal glucose level, thus allowing comparison of the latter to the arterial glucose level obtained from an arterial reference site elsewhere. This postulate is supported by the fact that increases in the portal glucose level decrease the hepatic vagal firing rate in the guinea pig and rat (24, 25) and that hepatic denervation abolishes the increase in net hepatic glucose uptake and hepatic glycogen deposition seen in the dog in response to portal glucose delivery (7).

The data from the present experiments confirm what has already been demonstrated in the dog (27, 28). Previous experiments (34, 35), however, did not extend this finding to the rat, because neither the hormone levels nor the glucose load to the liver was controlled. Nevertheless, Tordoff et al. (35), while examining the effect of the route of glucose infusion on food intake, also demonstrated that hepatic glycogen deposition was higher in rats receiving glucose into the hepatic portal vein, compared with the jugular vein. In their study, the insulin levels were similar regardless of the route of delivery, but the arterial and portal glucose levels were markedly different. As a result of their experimental design, the glucose load to the liver, although not calculated by the authors, was much higher during portal glucose delivery. Thus it remained unclear whether glycogen deposition was enhanced by

the portal signal or by the increased glucose load to the liver.

Shulman and Rossetti (34) compared the effects of intraduodenal and arterial glucose infusion on hepatic glycogen deposition in the rat. Hepatic portal and jugular vein blood glucose levels were measured at the end of each experiment. The blood glucose levels in the portal vein were similar in the two groups. Intraduodenal glucose administration induced greater hepatic glycogen deposition than did arterial glucose infusion, despite the fact that the load of glucose reaching the liver and the plasma insulin level were lower with the intraduodenal infusion. Whereas these data thus support the existence of the "portal signal" in the rat, they have two limitations. First, since the study design did not allow the investigators to clamp the pancreas, the venous insulin levels in the groups receiving glucose via the artery were almost twofold higher ( $370 \pm 61 \mu\text{U/ml}$ ) than in the group receiving glucose intraduodenally ( $196 \pm 32 \mu\text{U/ml}$ ). It has been demonstrated that the brain can sense physiological variations in the arterial insulin level (9, 10), and it is possible, therefore, that the high level of insulin within the brain limited glycogen deposition in the liver. Indeed, high brain insulin levels have been shown to increase sympathetic output under hypoglycemic conditions (10). Second, as discussed by Shulman and Rossetti (34), their results did not allow them to differentiate between a gut factor and a portal factor in triggering the increase in hepatic glycogen deposition. Our results, on the other hand, clearly point to a portal signal and do not have the confounding problem of differing insulin levels at the brain level. These two differences are very important because they allowed us to precisely isolate the portal signal per se, thereby making our results unique. It should be noted that our results do not allow us to determine whether the glycogen deposition occurred by the direct or indirect pathway. Earlier work in the dog would suggest that it is probably due to both pathways, as was suggested by Shulman and Rossetti (34).

In conclusion, the present study demonstrated that in pancreatic-clamped unrestrained, conscious rats portal glucose infusion causes a marked increase in hepatic glycogen deposition. This indicates that mechanistic studies on the regulation of the hepatic glycogen deposition by portal glucose delivery can be undertaken in the rat.

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