Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 7300–7304, October 1987
Medical Sciences

Insulin exerts metabolic and growth-promoting effects by a direct action on the liver *in vivo*: Clarification of the functional significance of the portal vascular link between the beta cells of the pancreatic islets and the liver

(insulin-like growth factor I/somatomedin C/body growth/diabetic rats)

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Communicated by Howard A. Bern, July 2, 1987 (received for review April 15, 1987)

ABSTRACT **The functional significance of the portal vascular link between the beta cells of the pancreatic islets and the liver has not been established. Previous studies indicated that insulin does not acutely regulate glucose metabolism by a direct hepatic effect. More recent observations suggest that the role of insulin in regulating body growth may be mediated, at least in part, by the liver. Our experiments were designed to test whether insulin can promote body growth and regulate glucose metabolism by a direct hepatic action *in vivo*. Rats were made diabetic by injections of streptozotocin, and insulin or solvent was infused into the jugular vein (JV) or the hepatic portal vein (HPV) for 14 days using catheters that were attached to osmotic minipumps. Infusion of a low dose of insulin (2 units per kg per day) into the JV had no effects on the hyperglycemia, body weight gain, tail growth, tibial epiphysial cartilage plate thickness, or serum levels of somatomedin C in the diabetic rats. However, the same dose given into the HPV caused a 30% reduction of blood glucose and stimulated a significant degree of growth, as determined by all indices. Infusion of a higher dose of insulin (5 units per kg per day) into either vein caused full restoration of body weight gain and tail growth and it restored the glycemic status almost to normal. However, it did not increase the tibial epiphysial plate width or serum somatomedin C levels above those of the rats given the low dose of the hormone into the HPV. These results indicate that insulin can act directly on the liver to promote body growth and to regulate glucose metabolism. The significance of direct delivery of insulin from the pancreatic beta cells to the liver may be as much for growth control as for glucose homeostasis.**

The functional significance of the portal vascular link between the pancreatic alpha cells and the liver has long been recognized, at least with respect to the mobilization of hepatic glycogen stores by glucagon. However, the possible importance of direct conveyance of insulin from the beta cells to the liver via the hepatic portal vein (HPV) has not been established. Numerous studies indicate that this vascular connection is not important for the metabolic actions of insulin. When the hormone was infused directly into the liver via the HPV, it was generally either no more or even less efficacious at changing glucose metabolism than when it was delivered into a peripheral vein (1–6).

Evidence from various sources indicates that insulin contributes to the regulation of body growth by several means, some of which may be hepatically mediated (7, 8). The growth retardation that occurs in many diabetic human and animal subjects is frequently accompanied by low serum levels of somatomedin C (Sm-C) (9–13). Restoration of growth of these subjects by insulin therapy is associated with a normalization of their serum insulin-like growth factor type I (IGF-I) concentration (9–13). More recently, Scheiwiller *et al.* (13) reported that infusion of purified human IGF-I into diabetic rats caused a significant degree of growth restoration.

The liver is generally considered to be the major source of serum Sm-C (14, 15), and insulin can stimulate hepatic cells to secrete that hormone *in vitro* (16–19) and in organ perfusion systems (20–22). Furthermore, insulin suppresses hepatic secretion of a factor that inhibits the effects of IGF-I (23). Thus it seems possible that the restorative effect of insulin therapy on growth in diabetic animals is accomplished in large part by a direct action of the hormone on the liver. However, several observations are inconsistent with that conclusion. In some human diabetic subjects serum IGF-I levels are not reduced (24–26) and in others they may even be elevated (27–29). Furthermore, Schalch *et al.* (30) found no effect of insulin on Sm-C secretion in a perfused rat liver system, and Scott and Baxter (11) reported that although insulin treatment of diabetic rats restored their serum Sm-C levels and the capacity of their hepatocytes to secrete the IGF *in vitro*, it failed to stimulate Sm-C secretion by hepatocytes of the diabetic rats *in vitro* when added to the incubation medium. Furthermore, several studies have shown that IGF-I can be secreted by a variety of cell types in addition to those of the liver (31–40), and insulin can stimulate them to secrete Sm-C *in vitro* (38, 39) and *in vivo* (41). Hence, insulin may maintain serum Sm-C levels and somatic growth by both hepatic and peripheral actions.

Despite these reservations, the bulk of the available evidence suggests that endogenous insulin may regulate somatic growth by directly stimulating hepatic secretion of IGF-I. Thus, the functional significance of the direct vascular link between the beta cell and the liver could be for somatic growth. However, as far as we are aware, it has not been shown that insulin can promote a significant degree of body growth by a direct effect on the liver *in vivo*. Accordingly, we have attempted to determine whether it can exert such an effect in diabetic rats. In addition, we were also interested in learning whether direct delivery of a low dose of insulin into the liver on a chronic basis would have significant metabolic effects in such rats even though acute experiments were generally negative in this regard (1–6). For these studies, we used a modification of the catheterization procedure developed by Mick and Nicoll (42) for delivery of hormones into the HPV.

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Abbreviations: GH, growth hormone; Sm-C, somatomedin C; IGF-I, insulin-like growth factor type I; JV, jugular vein; HPV, hepatic portal vein.

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MATERIALS AND METHODS

Male Long–Evans rats (body weight, 130–160 g) were obtained from our own breeding colony or from Simonsen Laboratories (Gilroy, CA). Husbandry conditions and anesthetics used were as described (43). Some of the rats were made diabetic by giving them an intrajugular injection of streptozotocin (65 mg per kg of body weight) on each of 2 consecutive days to destroy the beta cells of the pancreatic islets (44). Control rats were given intrajugular injections of the citric acid/saline solvent. Two days later, the urine of the rats was tested to determine whether they were diabetic. Glycosuria of 2% or greater was considered to be a positive indication of diabetes mellitus. The diabetic rats then received 30 units of insulin per kg of body weight per day (Iletin regular, 100 units/ml; Eli Lilly) subcutaneously in 2 daily injections for 4 days to enable them to survive the catheterization surgery on the 3rd day after beginning the insulin therapy.

Two weeks after catheterization, they were anesthetized and a blood sample was obtained by cardiac puncture for measurement of serum Sm-C levels by RIA using the acid/ethanol extraction method of Daughaday *et al.* (45). The antibody to human IGF-I was obtained from the National Hormone and Pituitary Program and the labeled Sm-C and purified human IGF-I used as a standard were provided by E. M. Spencer (Children's Hospital, San Francisco) (46). Glucose (47) and β-hydroxybutyrate (48) levels in the serum samples were also measured. The rats were then decapitated and the tibiae were removed for measurement of the thickness of the epiphysial cartilage plate (49), which is an index of their growth rate (49, 50). The body weight and tail length of each rat were recorded on the day of catheterization and when they were killed.

The rats received intravenous infusions of either 2 or 5 units of the insulin per kg of body weight per day or solvent, which was a variation of that described by Patel (ref. 51; 1.6% glycerol/0.02% sodium azide/0.7% L-glutamic acid). The infused solution also contained 10% (vol/vol, 1000 units/ml) sodium heparin. The doses of insulin used were based on the results of Patel (51), who found that it required ≈9 units per kg per day of insulin, delivered intraperitoneally by osmotic minipump, to normalize plasma glucose in diabetic rats.

Details of the procedures used to construct the catheters and to attach them to the osmotic minipumps were described (43). The methods used by Mick and Nicoll (47) to catheterize the jugular vein (JV) and the HPV of pigeons were modified for use in the rat. A 2-cm-long incision was made over the right JV extending from the clavicle toward the ear. The closed tip of a hemostat was gently inserted between the skin and the muscle layer over the thorax; the hemostat was then opened and removed, leaving a small pocket beneath the skin. The osmotic minipump with the catheter attached was inserted into this pocket, leaving the stretched PE 10 tubing exposed over the JV, which was isolated by gently removing the overlying connective tissue with toothed forceps. Two ligatures (000 surgical silk, ≈6 cm long) were slipped beneath the external branch of the JV for use in manipulating the vessel as well as for tying the catheter into place. A small hemostat was used to clamp the external JV superior to the ligatures and an insect pin was used to puncture the wall of the JV at a point just proximal to where the vessel was clamped. The stretched PE 10 tubing was inserted through the hole in the vessel and advanced until the tip of the catheter could be seen in the junction of the JV with the brachiocephalic vein. It was then secured with the two ligatures, which were reinforced with cyanoacrylate glue, and the incision was sutured closed and reinforced with wound clips.

The HPV was catheterized by making a 3-cm incision along the midline of the abdomen starting ≈2 cm below the thorax. The cecum was gently pulled out of the abdominal cavity, along with portions of the small intestine. A mesenteric vein draining the cecum was exposed by carefully removing the overlying connective tissue. Two ligatures (000 surgical silk, ≈6 cm long) were slipped beneath the vessel near the cecum. A small hemostat was used to clamp the vein on the distal side of the ligatures and an insect pin was used to puncture the vessel on the proximal side of the clamp. The tip of the pin was left inside the vein while the catheter was readied. As the insect pin was withdrawn, the stretched portion of the catheter was inserted into the vein and advanced to the point where the mesenteric vein joins the HPV. The catheter was tied into place using the two ligatures, which were placed under the vein. Additional ligatures were used if needed. They were reinforced with a drop of cyanoacrylate glue, and the minipump attached to the catheter was placed inside the abdominal cavity. The muscle layer was sutured, and then the skin incision was sutured and reinforced with metal wound clips.

RESULTS

The data in Fig. 1 show that the streptozotocin treatment caused severe nonfasting hyperglycemia and there were no significant differences among the groups infused with the low dose of insulin into the JV or those given the solvent in either vessel. However, infusion of the insulin (2 units per kg per day) into the HPV lowered serum glucose levels to ≈70% of that in the rats given solvent into the HPV ($P < 0.05$). None of the rats given either solvent or the low dose of insulin showed any difference in the severity of their glycosuria (≥2%) between the beginning and end of the experimental periods. The higher dose of insulin lowered the blood glucose levels to ≈40% of those in the respective solvent-infused groups but these animals were still slightly hyperglycemic. The β-hydroxybutyric acid levels were very low or undetectable in all of the diabetic rats, even in those not treated with insulin (data not shown). Thus, they had moderately severe nonketotic diabetes mellitus.

Increase in tail length was used as an index of skeletal growth (Fig. 2). In comparison to nondiabetic controls, the diabetic rats receiving solvent into the JV or the HPV showed

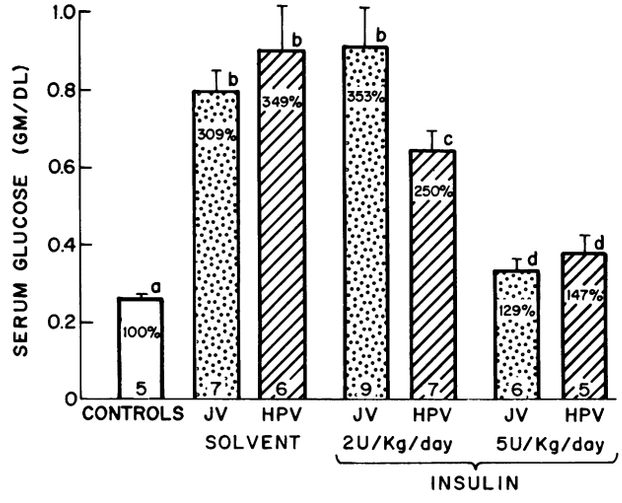

FIG. 1. Serum glucose concentration in nonfasted control and diabetic rats. The latter were infused with solvent or insulin into either the JV or HPV for 14 days. The number of animals in each group is given at the base of each column and the vertical lines above the top of each are the SEM. Columns superscribed by a common letter are not significantly different from each other, whereas those with a different letter are significantly different from each other at $P < 0.05$ or better.

growth inhibition of 72% and 77%, respectively. Infusion of insulin at 2 units per kg per day into the JV had no effect but the same dose given into the HPV increased tail growth to 57% of that of the nondiabetic rats and to 333% of that of animals receiving the solvent intrahepatically. The higher dose of insulin fully restored tail growth in both groups. The body weight gain of these groups, which is an index of somatic growth, paralleled the change in tail growth (data not shown).

Tibial epiphysial plate width measurements and serum Sm-C concentrations (Figs. 3 and 4, respectively) generally reflected the data on tail growth (Fig. 2). The rats given the low dose of insulin into the JV had plate widths and serum Sm-C levels similar to those of the animals given solvent in either vessel. However, the low insulin dose given into the HPV caused a significant increase in both of these parameters. The dose of insulin (5 units per kg per day) given by either route did not elevate serum IGF-I levels nor did it stimulate tibial epiphysial plate growth significantly more than the lower dose given into the HPV.

DISCUSSION

Our experiments clearly demonstrate that intrahepatic delivery of a low dose of insulin was highly effective at promoting growth, as assessed by several indices. The growth-promoting effects of the low dose of insulin were accompanied by partial correction of the metabolic derangements in the rats as the severity of their hyperglycemia was reduced (Fig. 1), but their glycosuria persisted. The effects of the intrahepatic insulin apparently were mediated by a direct action on the liver because infusion of the same low dose into the JV had no detectable effects, even on the sensitive tibial epiphysial plate. This conclusion is supported by the fact that infusion of the insulin into the HPV caused a striking increase in serum IGF-I levels (Fig. 4), which presumably mediated, at least in part, the somatotropic effects of the hormone. It seems likely that a reduction in hepatic secretion of a somatomedin inhibitor (23) would have been contributory to the growth response.

Although the high dose of insulin given by either route caused full restoration of body weight gain and tail growth and it reduced the glycemic status of the rats almost to normal, it did not restore serum Sm-C or tibial epiphysial plate width to levels equivalent to those in the control rats. Thus, it seems likely that factors other than IGF-I are involved in the growth response in these animals. The insulin treatment presumably reduced hepatic secretion of an inhibitor of IGF-I action (23) and it may act in concert with IGF-I to promote growth of peripheral tissues, as is suggested by the results of *in vitro* studies (52). It is of interest that in these animals the serum IGF-I concentrations are more consistent with their tibial epiphysial plate width measurements than with either weight gain or tail growth. This relationship may be due to the fact that the width of the cartilage plate is more closely related to the rate of growth at the time of death than to the amount of growth that occurred over any given time (49, 50).

The rats used in our experiments had diabetes mellitus of moderate severity, similar to those used by Maes *et al.* (12), in which there was no change in plasma growth hormone (GH) or hepatic GH receptor levels until 1 month of insulin deficiency. Accordingly, it seems unlikely that changes in either plasma GH or hepatic GH receptors contributed to the effects that we obtained with the low dose of insulin.

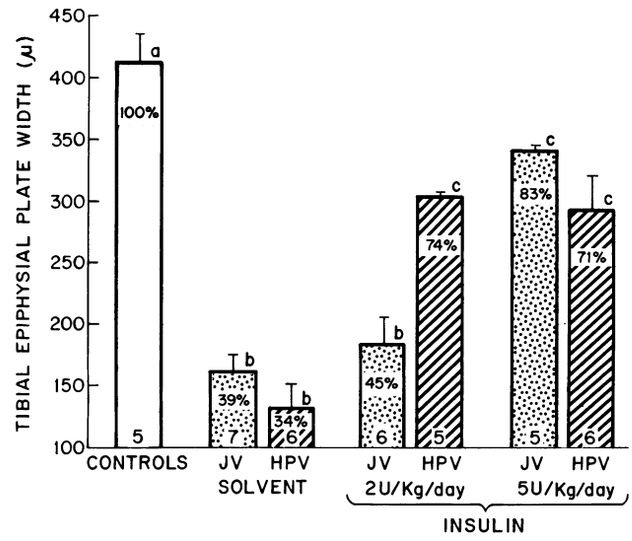

FIG. 3. Tibial epiphysial cartilage plate widths of rats described in the legend of Fig. 1.

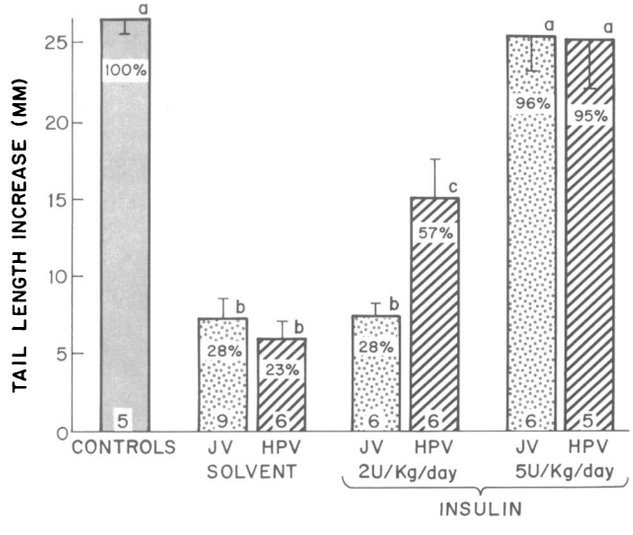

FIG. 2. Increase in tail length of rats described in the legend of Fig. 1.

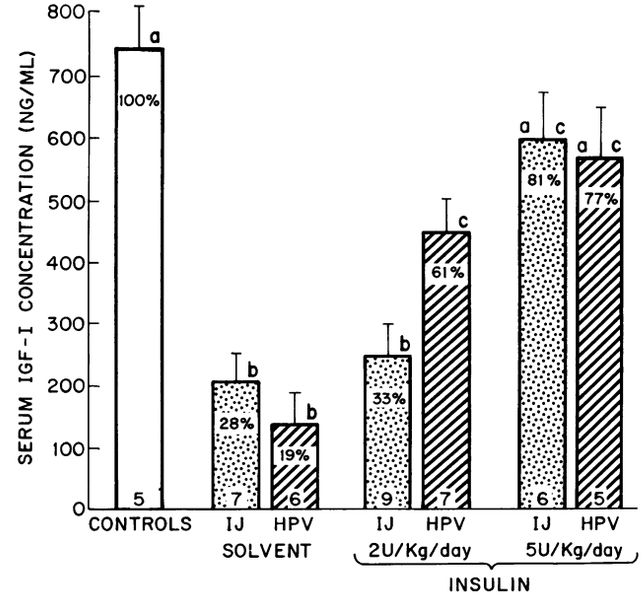

FIG. 4. Sm-C concentrations in serum of rats described in the legend of Fig. 1.

However, it is possible that insulin could increase hepatic responsiveness to GH by means other than changing GH receptor levels.

Heinze *et al.* (53) previously reported that endogenous insulin can have a growth-promoting effect in hypophysectomized rats. They used glibenclamide to stimulate insulin secretion and found that it restored serum levels of bioactive somatomedin almost to normal. However, the drug-treated rats showed no increase in body weight gain and only a small increase in serum insulin levels. Furthermore, the growth-promoting effects were slight and were only detectable by very sensitive indices, such as the tibia test. It is curious that despite the fact that the serum insulin concentration of these rats was only slightly increased, their circulating somatomedin levels were normalized, and yet their growth response was so meager (53).

Studies with transplanted pancreatic islets have shown that when they are placed in the liver or spleen of diabetic rats they are more effective at restoring growth and blood glucose concentrations than when placed in other sites (54–56). These results also suggest that insulin has important direct hepatic effects on growth and glucose metabolism. However, these findings (54–56) could simply be due to better survival of the transplants in the liver and spleen than in other sites.

Our finding of a preferential effect of intrahepatic delivery of the low dose of insulin on the glycemic status of the diabetic rats indicates that the hormone can have significant liver-mediated metabolic effects. This result differs from those of several acute experiments, which generally found that administration of insulin into the HPV was not more effective (or even less effective) at altering glucose metabolism than was its infusion into a peripheral vein (1–6). However, our findings suggest that the more significant aspect of insulin's direct action on the liver concerns the regulation of body growth. Hence, our results substantiate the previous studies that were suggestive of such a role of the hormone *in vivo* (see Introduction). Accordingly, the link between the beta cell and the liver via the hepatic portal system may be of significance for growth control as well as for metabolic regulation.

We are indebted to Dr. E. M. Spencer (Children's Hospital, San Francisco, CA) for the purified IGF-I preparations and to Drs. L. E. Underwood and J. J. Van Wyk (University of North Carolina, Chapel Hill) for the antiserum to somatomedin C. This work was supported by National Institutes of Health Grant HD-14661.

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