Effect of Insulin Infusion of Pancreas on Blood Glucose

John A. Colwell, M.D., and Arthur R. Colwell, Jr., M.D., Chicago

SUMMARY

Glucagon-free insulin in doses of 0.003 to 0.2 units per kilogram of body weight was infused into the pancreatic artery and portal vein during anesthesia or in fully recovered dogs. The changes in blood glucose concentrations were not significantly different between the two sites of infusion with any of the doses employed. These data indicate that insulin does not affect endogenous insulin secretion by a direct pancreatic mechanism. It may operate by reducing the blood glucose or via some other metabolic change. Similar experiments were conducted with glucagon (0.2 μg.) in anesthetized dogs. Glucagon infusion into the pancreas or liver failed to affect insulin or glucagon secretion as evidenced by peripheral venous glucose response. DIABETES *15*:123-26, February, 1966.

The concentration of glucose in arterial blood perfusing the pancreas is thought to be the major stimulus for insulin release.[1] However, a negative feedback mechanism responding to the arterial insulin concentration could regulate insulin secretion.[2] Supporting this idea is evidence that insulin administration reduced the concentration of insulin extractable from the pancreas,[3] inhibited islet growth,[4] caused beta cell degranulation[5] and temporarily impaired glucose tolerance[6] and decreased insulin liberation into the pancreatic vein.[7]

In order to study this mechanism, insulin was infused directly into the pancreatic circulation. The purpose was to inhibit insulin secretion by producing an increase in the concentration of *pancreatic* insulin, without the complicating effect of accompanying hypoglycemia[8] or other metabolic consequences of an elevated *systemic* blood insulin level. An inhibition of insulin secretion under these circumstances would be followed by a rise in peripheral blood glucose concentration. Small doses of glucagon were also infused in an attempt to assess the role of this hormone in control of pancreatic islet function. This experimental model was previously used successfully to demonstrate a direct action of the sulfonylureas in producing hypoglycemia by means of insulin stimulation.[9]

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From the Department of Medicine, Veterans Administration Research Hospital, Chicago, Illinois; the Department of Research and Education, Evanston Hospital, Evanston, Illinois; and the Department of Medicine, Northwestern University Medical School, Chicago, Illinois.

METHODS

Healthy dogs of both sexes weighing from 10 to 22 kg. were fasted from eighteen to twenty-four hours prior to the experiment. They were fed a standard diet containing 40 per cent carbohydrate, 35 per cent protein, and 25 per cent fat. Approximately one half of the experiments were performed on animals under anesthesia and the other half on animals after full recovery from the placing of catheters (done under anesthesia) several days previously. The former group of dogs was anesthetized with 25 mg. of sodium pentobarbital per kilogram of body weight. The right gastroepiploic artery was isolated and cannulated in a retrograde direction with a small polyethylene tube. Injection of the test solution permitted it to be carried by the normal arterial blood flow into the major portion of the pancreas. Anatomical considerations have been presented previously.[9] In the portal vein infusions, one of the branches of the mesenteric or splenic vein was cannulated. The catheter was placed so as to permit the infusion of the test solution directly into the liver along the vascular pathway followed by endogenous insulin or glucagon. In half of the anesthetized animals, the pancreas was infused first, shortly after the cannulas had been placed. Observations were made for ninety minutes after injection. Then an interval of thirty to sixty minutes was allowed before the same solution was introduced into the liver via the portal vein. The remaining acute experiments were done in reverse fashion, the liver being infused first and the pancreas last.

In the unanesthetized animals experiments were performed after recovery from the operation in order to avoid the effects of surgical stress and anesthesia on carbohydrate metabolism. Animals were fitted with catheters in the pancreatic artery or portal vein; each

catheter was exteriorized through a stab wound in the flank and fastened securely. Catheters were flushed with a small amount of 1 per cent heparin daily. With care the portal vein catheters stayed open as long as six weeks and the pancreatic artery catheters as long as two weeks after surgery.

Glucagon-free insulin or glucagon* was dissolved in 2.5 to 10 ml. of 0.9 per cent saline and infused at a rate of 0.5 to 1.0 ml. per minute. Table 1 gives the mean infusion rates and doses of insulin employed. The dose of glucagon employed was 0.2 μg. Blood was drawn into heparinized syringes from a peripheral vein at ten- to thirty-minute intervals before and after the beginning of each infusion. The concentration of glucose was measured in plasma samples by the glucose oxidase method[10] in the anesthetized animals and in whole blood by the method of Somogyi-Nelson[11] in the unanesthetized dogs. Plasma inorganic phosphorus concentration was estimated in a few animals by means of the method of Fiske and Subbarrow.[12]

RESULTS

In figure 1 the mean plasma glucose concentrations obtained following pancreatic and portal infusions in the anesthetized dogs are compared. Despite the wide range of insulin doses employed, pancreatic and portal infusions produced a similar degree of hypoglycemia. Statistical analysis by means of a group comparison showed no significant differences between the decrease in plasma glucose concentration in the two groups employing any insulin dosage. The decrease in plasma inorganic phosphorus was qualitatively similar following intraportal and pancreatic insulin administration.

In the unanesthetized dogs the maximum blood glucose fall was approximately equal in pancreatic and hepatic infusions of insulin in doses of 0.003 to 0.006 units per kilogram of body weight (figure 2). Although not shown here, several experiments using from 0.025 to 0.050 units of insulin per kilogram of body weight produced a fall in blood glucose qualitatively similar to those shown for the anesthetized dogs with these doses (figure 1). No rise in blood glucose concentration was seen, even at low doses.

Changes in plasma glucose concentration following glucagon administration into the pancreatic artery and portal vein are illustrated in figure 3. There was no significant difference between the two routes of infusion.

*Kindly furnished by W. R. Kirtley, M.D., Eli Lilly and Company, Indianapolis, Indiana. The glucagon-free insulin contained less than 0.01±0.003 per cent glucagon by weight.

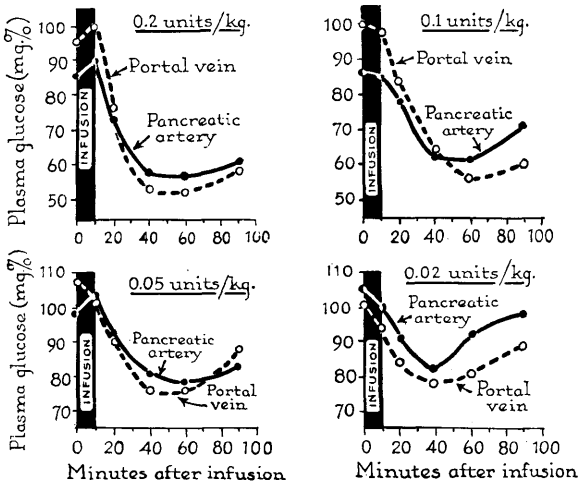

FIG. 1. Mean plasma glucose concentrations following the injection of small amounts of insulin into the pancreatic artery or portal vein.

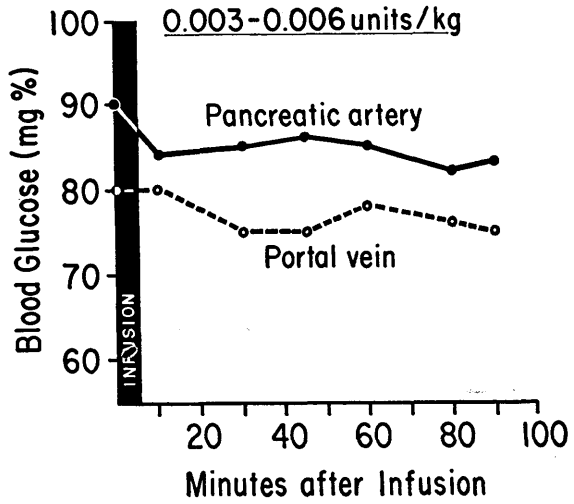

FIG. 2. Mean plasma glucose concentrations following the injection of small amounts of insulin into the pancreatic artery or portal vein in unanesthetized dogs.

DISCUSSION

It was our intention to inhibit the production or release of insulin by the beta cells by intrapancreatic infusion of small amounts of insulin. If the plasma insulin concentration acts as a homeostatic mechanism to control insulin secretion, then a pancreatic infusion of insulin should produce a less marked peripheral hypoglycemia than a portal infusion. If the proper dosage and rate of infusion are used, one would expect that pancreatic administration would be followed by a steadily *rising* blood glucose level as a reflection of a decreasing pancreatic insulin release. With the amounts

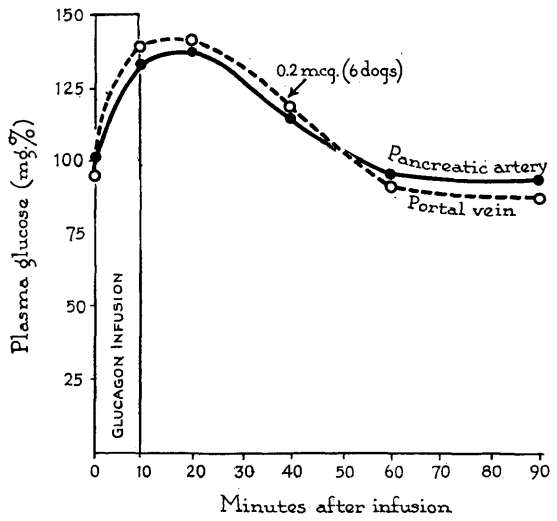

FIG. 3. Mean plasma glucose concentrations following the injection of 0.2 mcg. of glucagon into pancreatic artery or portal vein. Each point represents the mean value obtained in six experiments in anesthetized dogs.

TABLE 1

Mean doses and rates of insulin infusions into the pancreas and liver

Number of dogs	Anesthesia	Insulin U./kg. of body weights	Total units	mU./min.
4	+	0.2	2.70	270
4	+	0.1	1.50	150
4	+	0.05	0.65	65
4	+	0.02	0.27	27
4 (P.A.)* 5 (P.V.)†	0	0.003-0.006	0.04-0.12	8-24

*P.A. = Pancreatic artery
†P.V. = Portal vein

used, this failed to occur in either acute or chronic experiments. Insulin output was not measured directly, and it is possible that peripheral blood glucose would not reflect decreased insulin secretion.

In acute experiments in which insulin was administered intravenously to humans in amounts comparable to the smallest doses employed in our dogs, Samols and Ryder[7] found a decrease in endogenous insulin in the femoral artery after thirty to forty minutes. This was accompanied by a gradual fall in blood glucose, thought to be related to hepatic and tissue uptake of glucose. A lag period of at least ten minutes obtained before the effect was noted. These studies fail to localize the site of inhibition of insulin secretion to the pancreas, and the result could be secondary to the hypoglycemia produced by the insulin infusion.

In the present experiments, up to 2.7 U. of insulin were infused into the head and body of the gland. This represents approximately one third of the dog's daily insulin secretion. Assuming that the mean arterial concentration is 16 mU. per 100 ml. and the mean plasma flow through the pancreas is 6 ml. per minute,[1] then about 1 mU. of insulin normally perfuses the dog pancreas each minute. Since from eight to 270 times the estimated normal arterial level of insulin was added to the pancreas each minute (table 1), this amount represents a substantial excess of insulin per unit mass of beta cells. Yet no evidence of inhibition of insulin secretion was found. An insulin feedback mechanism could possibly function to inhibit insulin secretion only after the plasma insulin concentration has been elevated for a long period. The studies cited above[3-6] in which long-acting insulin was employed for a period of at least one week are in accord with this view. Since our infusions lasted only five to ten minutes, our data offer no new information on this possibility.

It appears that insulin secretion is not inhibited by an acute rise in pancreatic insulin concentration. Consideration of the available evidence indicates that hypoglycemia or one of its consequences inhibits insulin secretion.[1,3-8] Intrapancreatic glucagon administration also failed to influence peripheral blood glucose differently from intraportal infusion. This observation supports previous evidence that blood glucagon exerts no control over the release of glucagon from the pancreas.[13]

ACKNOWLEDGMENT

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The Fine Structure of the Early Atherosclerotic Lesion

(*Continued from page 114*)

mic reticulum, which could be interpreted as an indication of increased metabolic activity.

Beneath the endothelium, the fatty streak was characterized by the presence of many foam cells, the cytoplasm of which contained numerous small vacuoles which varied as much in staining quality as the endothelial cellular inclusions, but often had no limiting membrane. Such foam cells were often seen also on the luminal side of the endothelium with a projection inserted between endothelial cells, as though working their way through the endothelium. These cells in transit were seen much more often in the rabbit material than in the human. Still and Marriott found that monocytes (or lymphocytes) were present in greater numbers than had been reported previously, usually at the periphery of the lesion but sometimes forming the dominant cell type, and were prominent in the human fatty streak more often than in that of the rabbit. These often contained lipid inclusions, could also be found in apparent passage between endothelial cells and, to these investigators, formed transitional cells between the lymphocyte and the fully developed foam cell.

Fibroblasts and smooth muscle cells were "not always identified with confidence," but seemed to be present more often in less cellular portions of the lesion, deep to the endothelium. They both showed minor degrees of vacuolization and lipid inclusion, which were invariably simple and not as complicated as those seen in the foam cells. Both of these cell types were capable of producing intercellular materials such as collagen, a particulate granular material (which was not further identified), scant amounts of elastic tissue, and a fibrillary material. The latter was much more frequent in human lesions, as compared to the rabbit, and was thought to be fibrin, probably from the blood plasma. This reactive fibrosis was not notable in the vicinity of foam cells and did not seem to correlate with the presence of intracellular lipid.

These authors believe that the subintimal lipid of the fatty streak is derived both from circulating macrophages and all the normal cell types of the artery wall. From earlier work, they suspect that the macrophages, laden with lipid from the plasma, increase in number with increasing hyperlipemia. On the other hand, the fat appearing as inclusions in endothelial and medial cells may well arise from intracellular lipid synthesis. They are inclined to agree with others (H. Z. Movat, M. D. Haust, and R. H. More, Am. J. Path. 35:93, 1959) that plasma elements are also important in atherogenesis and may account for the intercellular fibrin.

Since these concepts are so crucial to an understanding of the atherosclerotic process, critical experiments are badly needed which will allow observation of the hypothesized course of a tagged lipid from plasma to endothelial and to subintimal cells. Then we might hope to identify the extent to which active cellular metabolic processes are involved in the build-up of the plaque, as well as in its degradation. In fact, the importance of plasma lipids and, hence, dietary fat in atherogenesis can only be placed on a firm basis when students of the fine structure of the arterial wall can reach agreement as to the sequence of changes in the morphological development of the fatty streak and their interpretation.

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