Lack of Hypoglycemic Activity of Insulin after Intestinal Administration to the Rabbit

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SUMMARY

The effect of insulin on blood glucose in the rabbit after direct instillation in the small intestine has been investigated. Crystalline Insulin, (U.S.P. U-40), administered via catheters placed in the duodenum, jejunum and ileum did not cause hypoglycemia at doses up to 50 U./kg. Tolbutamide (100 mg./kg.) produced a hypoglycemic response after oral and intestinal administration. The lack of response to insulin is believed to be due to the destruction of insulin by proteolytic enzymes of the gastrointestinal tract. DIABETES *14*:696-99, November 1965.

Since the original discovery of insulin by Banting and Best in 1921, many investigators have reported that insulin retains its hypoglycemic activity when administered orally with substances such as alcohol,[1-3] saponins,[4] oxgall,[5] resorcinol,[6,7] or quinine.[8,9] Many of these findings, however, were not observed by other investigators[10-14] and are now of historical interest only. A review of these and a variety of other agents which allegedly either aid the absorption or prevent biological inactivation of orally co-administered insulin has been presented by Hanzlik and Cutting.[8] The failure of insulin to induce hypoglycemia when administered orally is attributed to the breakdown of the hormone by the gastric and pancreatic proteinases.[15]

Recently Speth and Christian[16] reported that insulin, administered by the jejunal and ileal routes, elicited hypoglycemia equivalent to that obtained after intramuscular administration. The authors used rabbits with gastric, jejunal and ileal fistulas and "insulin was infused directly into the exposed portions of the gastrointestinal tract." These interesting findings prompted an investigation into the hypoglycemic activity of insulin injected into various sites of the gastrointestinal tract via indwelling catheters.

MATERIALS AND METHODS

Young male and female New Zealand rabbits, 1,100 to 1,600 gm., were maintained in an air-conditioned building in individual wire mesh hanging cages with free access to food pellets and water. Indwelling catheters were placed in the duodenal, jejunal and ileal portions of the intestine of all rabbits by the following technic. A midline abdominal incision was made on rabbits anesthetized with sodium pentobarbital (30 mg./kg.). An area in the duodenal portion of the intestine, one and one-half inches caudal to the pylorus, was penetrated with a 12 gauge, 1 in. needle. Polyethylene chloride tubing, of an internal diameter of .042 cm. (Suprenant Manufacturing Co., Boston, Mass.) was passed through the needle until approximately 2 cm. lay in the lumen of the duodenum. The needle was removed, leaving the tubing in place. A "purse string" suture was then placed through the muscular coat around and adjacent to the entrance site of the tubing, to impede extravasation of duodenal contents. Approximately 2 cm. cranially to the entrance site, the tubing was anchored to the intestine (single suture), care again being taken not to penetrate the intestinal lumen. Five milliliters of physiologic saline was then administered via the tubing and the operated area checked for leakage. This same technic was employed for placing properly identified tubing in the jejunal and ileal portions of the intestinal tract. A No. 1, 7½ inch Patterson trocar with cannula was then inserted through a small skin incision, located in the atlas area, and directed caudally, under the skin, until it reached an area 1 to 2 in. lateral to the abdominal incision; at this point, the abdominal wall was punctured and the trocar withdrawn, leaving the cannula in situ. The three free ends of the tubes were passed through the cannula until they emerged at the atlas area. The cannula was then removed. Sufficient slack was allowed so that movement of the animal would not displace the tubing. The abdominal and skin incisions were closed with mattress sutures. The exteriorized tubes were flushed with saline and plugged with wire pins marked to identify each route. The tubes were taped against the shaved portion of the neck area to prevent retraction. Each rabbit then received 60,000 units of procaine penicillin in oil, intramuscularly. All rabbits recovered uneventfully; a seven- to ten-day period was allowed

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before animal experimentation was carried out. All tests were performed after an eighteen-hour fast.

Crystalline Insulin, (U.S.P. U-40) was diluted in glass containers with physiologic saline so that any one dose was contained in a volume of 0.5 ml. Tolbutamide* was dissolved in a minimum amount of 1N NaOH and brought to a concentration of 100 mg./ml. with physiologic saline. These substances were administered through a 22 gauge needle connected to each tube which was then flushed with 1 ml. of saline. Gastric administration was via stomach tube. Blood samples were obtained from a marginal ear vein and glucose concentrations determined with the Technicon AutoAnalyzer.

RESULTS

A preliminary experiment was performed in which insulin was administered subcutaneously to the surgically prepared rabbits. The hypoglycemic effects of insulin given at 0.5, 1.0 and 2.0 U./kg. body weight are presented in table 1. All doses elicited good hypoglycemic responses. Blood glucose concentrations returned to normal by the seventh hour. On the basis of these results, a dose of 0.75 U./kg. body weight was selected for the studies in which insulin was to be administered by the subcutaneous, oral, duodenal, jejunal or ileal routes. The design utilized in the first experiment was a $2 \times 5 \times 3$ Youden Square[17] (two rabbits for each of the five routes, repeated three times, one week apart). Administration of insulin other than by the subcutaneous route had no effect on blood glucose concentrations. A second experiment (upper half of table 2) was carried out in which doses were increased to 2.25, and 4.50 U. insulin/kg. body weight. At these doses, Speth and Christian[16] reported hypoglycemic effects after intestinal administration of the hormone. The statistical design for this experiment was a 3×3 balanced lattice design[17] in which four animals were used for each of the nine treatments. Again, only the 0.75 U. dose, administered subcutaneously, elicited hypoglycemia. Also shown in table 2 (lower half) are the results of an experiment in which the doses of insulin were increased to 50 U./kg. As above, there were no hypoglycemic responses even at this high dose.

To determine the ability of the test system to respond

* Obtained from the Upjohn Co., Kalamazoo, Michigan.

TABLE 1

Blood glucose concentrations following a single subcutaneous or intraperitoneal administration of insulin to rabbits

Route	Dose U./kg.	Number of rabbits	Average blood glucose (mg./100 ml.) ± S.D. 0-hour	2-hour	4-hour	7-hour
S.C.	0.5	3	90 ± 7.1	51 ± 6.5	69 ± 11.2	88 ± 13.0
S.C.	1.0	3	92 ± 8.5	36 ± 5.3	56 ± 18.3	72 ± 31.3
S.C.	2.0	3	75 ± 1.8	31 ± 1.2	46 ± 7.1	89 ± 14.2
I.P.	0.75	5	91 ± 10.2	45 ± 12.8	93 ± 15.3	111 ± 4.9

TABLE 2

Blood glucose concentrations of rabbits following a single administration of insulin by several routes and doses

Route	Dose U./kg.	Number of animals	Average blood glucose (mg./100 ml.) ± S.D. 0-hour	1-hour	2-hour	4-hour	7-hour
Subcutaneous	0.75	4	85 ± 10.4	48 ± 3.7	51 ± 14.8	79 ± 29.2	102 ± 10.1
Oral	2.25	4	86 ± 8.4	82 ± 18.8	87 ± 9.5	98 ± 5.6	106 ± 5.5
Duodenum	2.25	4	86 ± 12.9	95 ± 11.0	96 ± 12.7	97 ± 9.2	102 ± 2.6
Jejunum	2.25	3*	86 ± 8.0	91 ± 1.7	98 ± 2.4	101 ± 3.1	110 ± 7.6
Ileum	2.25	4	86 ± 14.0	88 ± 9.0	92 ± 9.0	91 ± 9.6	103 ± 3.9
Oral	4.50	4	90 ± 8.2	96 ± 10.3	93 ± 4.5	99 ± 7.4	102 ± 11.5
Duodenum	4.50	4	85 ± 11.2	87 ± 3.6	89 ± 6.6	88 ± 10.0	102 ± 4.4
Jejunum	4.50	4	87 ± 10.3	94 ± 9.2	89 ± 9.4	98 ± 9.6	104 ± 6.3
Ileum	4.50	4	81 ± 2.2	89 ± 3.0	92 ± 3.0	95 ± 2.2	107 ± 6.4
Oral	50	2	89 ± 5.3	89 ± 1.7	89 ± 2.6	91 ± 1.7	90 ± 3.5
Duodenum	50	2	96 ± 3.5	89± 3.5	95 ± 2.0	92 ± 1.7	89 ± 5.3
Jejunum	50	2	83 ± 6.2	82 ± 21.2	92 ± 0.8	99 ± 2.0	101 ± 0.9
Ileum	50	2	83 ± 9.7	84 ± 5.3	90 ± 1.7	91 ± 0.8	84 ± 30.1

*Missing samples

to an orally active hypoglycemic agent, tolbutamide dissolved in the minimum amount of NaOH for solubilization was administered at a dose of 100 mg./kg., by the oral, duodenal, jejunal and ileal routes. The dose was contained in a volume of 3.0 ml. of saline. It will be observed (table 3) that tolbutamide caused hypoglycemia by all routes of administration. It appears, moreover, that the hypoglycemic responses to tolbutamide administered by the intestinal routes were equivalent to the response obtained after oral administration.

DISCUSSION

Speth and Christian[16] have concluded that once insulin has bypassed the stomach it is absorbed through the jejunum and ileum and exerts hypoglycemic effects grossly equivalent to those obtained after intramuscular administration. However, other investigators have reported on the lack of hypoglycemic effects of insulin when administered to animals by intestinal routes. Hypoglycemia was not observed by Laskowski, et al.[18] in ten experiments when insulin (40 U./kg. and 250 U./kg.) was injected into ligated jejunal loops of rats. Danforth and Moore[19] also failed to observe a response from insulin (800 U./kg. and 1,600 U./kg.) when injected into the ligated jejunal loops of rats. Moreover, Bollman and Mann[20] were unable to produce hypoglycemia in dogs following administration of large doses of insulin into duodenal, jejunal and ileal fistulae.

Since Speth and Christian[16] performed their studies in the rabbit, there exists the possibility of a species difference in the intestinal absorption of insulin. The results of our studies, however, would tend to rule out such an explanation. The lack of agreement in results from these two laboratories may, therefore, be a consequence of the different operative technics employed. It is conceivable that by exposing portions of the gastrointestinal tract to the external environment via fistulae,[16] *p*H, bacterial flora and most important, enzyme activity might be sufficiently affected so that biological inactivation of insulin does not occur. It has been shown that when enzyme activity is inhibited, insulin administered by the intestinal route exerts a hypoglycemic effect. Laskowski et al.[18] administered "pancreatic trypsin inhibitor" with insulin and obtained hypoglycemia. Danforth and Moore[19] also obtained a hypoglycemic response with insulin when trypsin and chymotrypsin activities were destroyed with .075 mg. diisopropylfluorophosphate administered into the intestine.

That the integrity of the insulin molecule must be maintained for biological activity, as suggested by Sanger,[21] is well known. Nicols[22] showed that removal of the terminal alanine and the C-terminal asparagine from insulin with carboxypeptidase reduced the activity of the product to 10 per cent of that of ox insulin as determined in the fasted rabbit. Treatment of ox insulin with trypsin produced alanine, a heptapeptide and the residue molecule (desheptapeptide alanine insulin, DHA insulin). Using both fasted rabbits and the rat diaphragm technic, Nicols[22] found DHA insulin retained 15 per cent of the biological activity of ox insulin; the heptapeptide exhibited no insulin-like activity. Carpenter and Baum[23] using fasted rabbits and a mouse convulsion test found no insulin-like activity from the heptapeptide and less than 2 per cent of the activity of insulin was obtained with DHA insulin. In addition, they reported that the rate of destruction of insulin with trypsin and the rate of loss of biological activity followed the same first order reaction, with 50 per cent destruction after approximately fourteen minutes. Laskowski et al.[18] showed a 50 per cent reduction in insulin activity after nine minutes incubation with trypsin.

The lack of hypoglycemic effects of insulin, observed in our studies after administration by various intestinal routes under quasinormal physiological conditions, is in agreement with the present concepts of insulin inactivation by gastrointestinal proteinases.

TABLE 3

Blood glucose concentrations of rabbits following a dose of 100 mg./kg. of tolbutamide by various routes

Route	Number of rabbits	Average blood glucose concentrations (mg./100 ml.) 0-hour	1-hour	2-hour	4-hour	7-hour
Oral	3	91 ± 8.0*	72 ± 17.6	42 ± 11.3	61 ± 19.6	55 ± 16.0
Duodenum	3	88 ± 5.8	58 ± 8.6	53 ± 13.3	62 ± 14.6	53 ± 5.5
Jejunum	3	87 ± 10.0	64 ± 2.0	67 ± 5.1	60 ± 16.5	59 ± 12.5
Ileum	2	88 (88-89)†	67 (64-70)	64 (63-65)	59 (55-64)	64 (48-80)

* Average ± S.D.
† Average and range

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Idiopathic Hemochromatosis and Bantu Siderosis

Recently, there have been statements that idiopathic hemochromatosis is simply a variant of the hemochromatosis found in conjunction with nutritional cirrhosis. It has been suggested that subjects suffering from some type of nutritional cirrhosis who are also exposed to large iron intakes develop hemochromatosis (R. A. MacDonald, Arch. Int. Med. *107*:606, 1961). If these two disorders are indeed similar, and if in idiopathic hemochromatosis there is no metabolic disorder, one would assume that histological findings in patients of both types would also be similar.

Animal studies have shown that pathologic changes present in rats fed corn diets enriched with iron are much like those found in persons suffering from siderosis due to excess iron intake. Distribution of iron within the tissues also is quite similar (Nutrition Reviews *22*:20, 1964; T. Gillman, M. Hathorn, and P. A. S. Canham. Amer. J. Path. *35*:349, 1959).

T. H. Bothwell, C. Abrahams, B. A. Bradlow, and R. W. Charlton have recently made a comparative histological study of thirteen individuals with idiopathic hemochromatosis and thirteen Bantu patients with hepatic siderosis (Arch. Path *79*:163, 1965). Many studies have indicated that the Bantu may ingest 50 to 100 mg. of iron each day, and that much of this is consumed in alcoholic drinks. Other nutritional problems may also lead to liver disease, so that the factors related to the development of cirrhosis may be complex. However, the evidence suggests that severe siderosis is almost always found in the Bantu, who has had a long exposure to alcoholic beverages produced locally and known to contain large amounts of iron (H. C. Seftel et al., J. Lab. Clin. Med. *58*:837, 1961).

The patients suffering from idiopathic hemochromatosis all had liver biopsies. Liver biopsy was carried out

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