Glucagon Release from Isolated Pancreas in Streptozotocin-treated Rats

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SUMMARY

Isolated islets (collagenase digestion) and pancreas pieces were prepared from control and insulin-deficient (streptozotocin-treated) rats. Results of experiments were similar with the two pancreas preparations. Glucagon release was increased from islets which were severely insulin-deficient but not from islets showing only moderate insulin deficiency. The normal suppressive effect of glucose on glucagon release was also abolished in the severely insulin-deficient islets. It is suggested that the stimulatory effect of insulin deficiency on glucagon release may be mediated through glucose deprivation in the alpha cell and an alteration in tissue cyclic AMP levels. DIABETES *22:* **797-800, November, 1973.**

Unger and co-workers[1] have reported relative hyperglucagonemia in patients with diabetes mellitus. The disorder is also seen in dogs rendered insulin-deficient with alloxan.[2] It is possible that the hyperglucagonemia may be a consequence of insulin deficiency.

Streptozotocin is cytotoxic for the beta cells of the pancreatic islets, but the alpha cells remain unchanged;[3] as the effect of the drug is dose-related, a graded series of insulin-deficient states can be produced. The present studies examine the effect of streptozotocin treatment on glucagon and insulin release from isolated pancreas preparations in the rat.

METHODS AND MATERIALS

Wistar rats weighing 150 to 200 gm. at the time of injection were utilized. Each was given a single intravenous or intraperitoneal injection of streptozotocin (Lot No 9164-UPV-59, kindly supplied by the Upjohn Co, Kalamazoo, Mich.) in a dose of 40 to 75 mg./kg. body weight, dissolved immediately beforehand in a citrate-phosphate buffer pH 4.5. At the same time rats of similar weight were put aside to be used as controls in the experiment. Animals in both groups were maintained on a normal diet for periods varying from three to six weeks and were then decapitated in the fed state before removal of the pancreas. Blood sugar levels at death were estimated with an AutoAnalyzer.

For the hormone release studies, two methods of pancreas preparation were used. In the first, isolated islets were prepared by collagenase (Kochlite Laboratories) digestion according to the method of Vance, Buchanan, Challoner and Williams.[4] Islets from streptozotocin-treated animals appeared paler than control islets under the dissecting microscope and they sedimented more slowly during preparation. Islets were grouped in ten and the amount of hormone release was expressed per ten islets per thirty minutes of incubation. In the second method, 2 to 5 mm. pieces of pancreas were incubated in the presence of excessive glucagon antibody according to the method of Malaisse and Malaisse-Lagae[5] as modified by Zandomeneghi and Buchanan.[6] The amount of hormone released was expressed per milligram of dried pancreas weight. All incubations were performed in the presence of Trasylol 1000 KIU/ml. (kindly supplied by Bayer) to prevent breakdown of glucagon.

Insulin was measured by radioimmunoassay using a charcoal separation technic[7] and human insulin (MRC) as standard. Glucagon was measured by modifications of a radioimmunoassay method[8] and also using a charcoal separation technic.[7]

RESULTS

The effect of streptozotocin on weight, blood sugar and plasma insulin. Streptozotocin in a dose of 65 to 75 mg./1 kg. intravenously invariably produced a severe

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Accepted for publication September 4, 1973.

diabetic state with wasting, polyuria and gross hyperglycemia. Doses of 40 to 55 mg./1 kg. resulted in milder degrees of diabetes. The intraperitoneal route gave similar but more variable results. The diabetic rats were divided into those with moderate diabetes (blood sugar level at death 150 to 300 mg. per cent) and severe diabetes (blood sugar level greater than 300 mg. per cent). Control rats had blood sugar values of 140 ± 4 mg. percent (mean ± S.E.M.), gained weight at the rate of 34± 5 gm. per week and had plasma insulin determinations of 24 ± 3 μU/1 ml. Moderately diabetic rats had blood sugar values of 188 ± 8 mg. per cent, a weight gain of 15 ± 3 gm. per week and plasma insulin levels of 16± 3 μU./1 ml. In severely diabetic animals blood sugar was 503 ± 12 mg. per cent, weight *loss* was 11 ± 2 gm. per week and plasma insulin was 5 ± 1 μU./1 ml. Plasma glucagons were not recorded because of technical difficulties in the plasma assay.

The effect of streptozotocin in insulin release. The insulin released from isolated islets (collagenase) and pancreas pieces is shown in tables 1 and 3 respectively. In control rats highly significant amounts of insulin were released in the presence of 300 mg. per cent glucose as compared with 30 mg. per cent in both isolated islets and pancreas pieces preparations. In streptozotocin-treated rats in which blood sugar at death ranged from 150 to 300 mg. per cent (moderate diabetes), a still significant increase ($p < 0.025$ in isolated islets preparations) was noted, but the amounts of insulin at the 30 and 300 mg. per cent glucose incubation were significantly reduced from the control preparations ($p < 0.05$ and $p < 0.0025$, respectively). In streptozotocin-treated rats with blood sugar levels at death greater than 300 mg. per cent (severe diabetes), no significant increase was seen in insulin release at 300 mg. per cent as compared with 30 mg. per cent in both the isolated islets and pancreas pieces. The insulin release in the severely diabetic animals was significantly reduced from that in both the control and moderately diabetic rats in the isolated islet preparations, and from the control rats in the pancreas pieces preparations.

The effect of streptozotocin on glucagon release. The effect of streptozotocin treatment on glucagon release is shown in table 2 for the isolated islets and in table 3 for the pancreas pieces. In control rats, both in the isolated islet and pancreas pieces preparations, more glucagon was released at 30 than at 300 mg. per cent glucose, but the difference achieved significance only in the isolated islets preparations. In rats with moderate diabetes the glucagon release was also significantly greater at 30 than at 300 mg. per cent glucose (isolated preparation). However, in severely diabetic rats the glucagon release pattern was reversed in that more glucagon was released at 300 mg. per cent than at 30 mg. per cent ($p < 0.0025$ for the pancreas pieces, but not significant in the isolated islet preparations). In

TABLE 1
Insulin release from isolated islets of control and diabetic rats

	Insulin release (μU./10 islets/30 min.)		P value† (30 vs. 300 mg.%)
Glucose concentration, mg.%	30	300	
Control rats	106±9(75)*	244±13(70)	<0.0005
Rats w/moderate diabetes‡	71±13(20)	153±35(19)	<0.025
Rats w/severe diabetes	33±6(74)	37±7(74)	n.s.
P value, control vs. moderate	<0.05	<0.0025	
P value, moderate vs. severe	<0.005	<0.0005	
P value, control vs. severe	<0.0005	<0.0005	

Figures are mean ± S.E.M.
*Figures in parentheses refer to number of incubates.
†P values from Student's *t* test.
‡For definition of moderate and severe diabetes see text.

TABLE 2
Glucagon release from isolated islets of control and diabetic rats

	Glucagon release (ng./10 islets/30 min.)		P value† (30 vs. 300 mg.%)
Glucose concentration, mg.%:	30	300	
Control rats	1.73±0.17(75)*	1.34±0.14(70)	<0.05
Rats w/moderate diabetes‡	1.93±0.31(20)	1.42±0.15(20)	<0.05
Rats w/severe diabetes	2.04±0.19(59)	2.24±0.23(57)	n.s.
P value, control vs. moderate	n.s.	n.s.	
P value, moderate vs. severe	n.s.	<0.025	
P value, control vs. severe	n.s.	<0.001	

Figures are mean ± S.E.M.
*Figures in parentheses refer to number of incubates.
†P values from Student's *t* test.
‡For definition of moderate and severe diabetes see text.

TABLE 3
Insulin and glucagon release from pancreas pieces in control and diabetic rats

	Insulin release (μU./mg./30 min.)		P value† (30 vs. 300 mg.%)	Glucagon release (ng./mg./30 min.)		P value (30 vs. 300 mg.%)
Glucose concentration, mg.%:	30	300		30	300	
Control rats	67±12(33)*	149±19(34)	<0.0005	1.46±0.18(31)	1.36±0.21(33)	n.s.
Severely diabetic rats‡	19±6(22)	32±8(22)	n.s.	2.63±0.39(20)	4.50±0.43(22)	<0.0025
P value, control vs. severe	<0.0025	<0.0005		<0.0025	<0.0005	

Figures are mean ± S.E.M.
*Figures in parentheses refer to number of incubates.
†P values from Student's *t* test.
‡For definition of severe diabetes see text.

addition highly significantly greater amounts of glucagon were released in this group of rats than the control rats at 300 mg. per cent glucose in the incubate ($p < 0.001$ for the isolated islets; $p < 0.005$ for the pancreas pieces), and at 30 mg. per cent glucose in the incubate, but the difference achieved significance ($p < 0.0025$) only in the pancreas pieces preparations.

DISCUSSION

The experiments were conducted in two pancreas models as glucagon release from isolated islet preparations has been found to be more variable than insulin release.[4] The more variable glucagon release in the isolated islet preparations may be due to damage, during the collagenase incubation, occurring in the alpha cells, which are situated more peripherally in the rat islet than the beta cells. Glucose stimulation of insulin release was satisfactorily demonstrated in both models. Glucagon release in control rats was significantly suppressed by high glucose concentrations in isolated islets but not in pancreas pieces, and a similar effect of diabetes on glucagon release was seen in both preparations, although it was more striking in the pancreas pieces. It is concluded that both preparations are satisfactory, although each has its own particular advantages. The isolated islets (collagenase preparation) are prepared virtually clean of exocrine tissue; therefore, by use of beta cell toxins such as streptozotocin, a largely glucagon cell preparation can be prepared, which would be eminently suitable for metabolic studies. The pancreas piece preparation is simpler to prepare and, therefore, less subject to damage. Damage to released glucagon can be prevented by Trasylol and excessive glucagon antibody. For simple release studies, the pancreas pieces would appear to be a convenient model.

Streptozotocin in graded doses can produce insulin deficiency states of varying degree. Thus, some animals that received streptozotocin exhibited only moderate hyperglycemia (150 to 300 mg. per cent blood sugar), and the islets of these animals continued to show an insulin response to glucose, whereas the islets of animals with marked hyperglycemia (> 300 mg. per cent blood sugar) showed no insulin response to glucose. These different levels of insulin deficiency are analagous to the spectrum of insulin deficiency in human diabetes mellitus.

Glucagon release was not altered from the control situation until the animal's islets were severely insulin deficient and showed no insulin response to glucose stimuli. The islets from severely insulin-deficient rats exhibited excessive glucagon release, particularly at high glucose concentrations. A similar effect of streptozotocin on rats has been noted on blood glucagon levels.[9] Howell, Edwards and Whitfield[10] were, however, unable to show abnormal glucagon release patterns in guinea pigs treated with streptozotocin. However, their animals were studied five to six days after treatment and were not hyperglycemic at death, although their islets were insulin deficient. Also guinea pigs may be somewhat resistant to streptozotocin.[11] Muller, Faloona and Unger[2] have reported hyperglucagonemia in dogs made insulin deficient with alloxan. In clinical diabetes mellitus only relative hyperglucagonemia is noted, although marked hyperglucagonemia is noted in severe ketoacidosis, when insulin deficiency is presumably marked. It might also be suggested that abnormal glucagon metabolism seen in the human diabetic state[1] may be not a primary defect but secondary to insulin deficiency.

The mechanism by which insulin deficiency stimulates glucagon release is not clear. Since glucagon stimulates insulin release,[12] it would be logical, when

there is insulin lack, that insulinotropic substances such as glucagon may be secreted. The release of glucagon may result from a state of glucose deprivation in the alpha cell consequent to the lack of sufficient insulin to transport glucose.

Under normal physiologic conditions insulin exerts a continuous damping effect on tissue levels of cyclic AMP.[13] Cyclic AMP may regulate the secretion of glucagon[14] and, therefore, when there is insufficient insulin to reduce cyclic AMP levels within the alpha cell, glucagon may be secreted as a consequence. The suppressive effect of glucose on glucagon release[4] would appear to be lost under these conditions.

ACKNOWLEDGMENT

This work was supported by grants from the Medical Research Council and The British Diabetic Association.

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