Effects of Hyperglycemia, Tolbutamide and Glucagon on the Pathways of Glucose Oxidation in the Goosefish Islet in Vitro

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SUMMARY

The contribution of the pentose and Krebs cycles to glucose metabolism has been determined in the isolated goosefish islet, in vitro, using glucose-2-C-14. Hyperglycemia caused increased net glucose metabolism by both the pentose and Krebs cycles, while glucagon and tolbutamide had no significant effect on net glucose metabolism by either pathway. The rate of reduced pyridine nucleotide formation was calculated and was increased with hyperglycemia but not with tolbutamide or glucagon. DIABETES *19*:554-58, August, 1970.

The release of insulin stimulated by glucose depends on the further oxidation of glucose-6-phosphate. Mannoheptulose and D-glucosamine, which interfere with phosphorylation of glucose, decrease or abolish insulin release in response to hyperglycemia.[1-3] Islet tissue incubated in a nitrogen-carbon dioxide atmosphere or with 2,4 dinitrophenol does not release insulin in response to hyperglycemia.[4] The release of insulin induced by glucose occurs within seconds and ceases quickly after removal of the stimulus.[5] A metabolic product of glucose or a cofactor related to its metabolism is thought to be responsible for initiating the release of insulin from islet tissue.[6] On the other hand, glucagon has been shown to cause insulin release in the absence of glucose in the medium[5,7] and the insulin release caused by tolbutamide is not inhibited by mannoheptulose.[4] These substances may exert an effect independent of glucose metabolism.

The penitols, xylitol and ribitol, and the five carbon sugar ribose have been shown to be effective in causing insulin release from isolated rat islets, presumably by entering the pentose phosphate pathway.[8] In addition, 20 mM. glucose, tolbutamide, citrate, glucagon and theophylline have been reported to increase the intracellular concentration[9] of an intermediate in the pentose cycle, 6-phosphogluconate. Montague and Taylor have suggested on the basis of the above data that glucose is metabolized by the pentose cycle to stimulate the secretion of insulin.[9]

Islet of Langerhans tissue from the goosefish synthesizes and stores insulin[10,11] and hyperglycemia causes insulin release from toadfish islet, in vitro.[12] NADH, NADP, and NADPH have been shown to stimulate the release of insulin from toadfish islet, in vitro.[13] Watkins has recently observed that tolbutamide and glucagon, in concentrations identical to those used in this study, caused significant release of insulin from toadfish islet, in vitro.[14]

In order to examine the possibility that the pentose cycle and/or formation of reduced pyridine nucleotides is related to insulin release by islet, we have studied the effect of hyperglycemia, tolbutamide and glucagon on the metabolism of glucose by the pentose and Krebs cycles. Calculations have been made of the rate of formation of NADH and NADPH.

MATERIALS AND METHODS

Goosefish (*Lophius piscatorius*), captured off Nantucket Island in the months of July and August, 1967 and 1968, were maintained in tanks of circulating sea water at 18° C. Within twenty-four hours of their capture, the fish were stunned with a blow on the head and the principal islets removed as previously described.[15]

Incubations were carried out for two hours in a modified Krebs Ringer bicarbonate buffer with either glucose-2-C-14 or glucose-6-C-14 in the medium as previously described. Radioactive compounds were purchased from the New England Nuclear Corporation, Boston, Massachusetts. Crystalline pork glucagon (Eli Lilly and Company, Indianapolis, Indiana) and tolbutamide (Upjohn Company, Kalamazoo, Michigan) were added to the mediums in concentrations of 5 μg./ml. and 200 μg./ml., respectively. The concentration of glucose

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was 0.25 mg./ml. in the control flask as well as those flasks containing glucagon and tolbutamide; in the remaining flask the glucose concentration was 2.00 mg./ml. The volume of the medium was 0.5 ml. in the experiments with glucose-2-C-14 and 0.2 ml. with glucose-6-C-14. The tissue weights ranged from 12 to 25 mg.

The incubations, the determinations of the distribution of C-14, and the measurements of the specific yield of C-14-O_2 from glucose-6-C-14 were done as previously described,[15,16] except that the uptake of glucose was measured in duplicate by a glucose oxidase method.

Calculation of Krebs cycle oxidation of glucose carbon was made according to Katz and Wood[17] and Rognstad and Katz[18] using glucose-6-C-14. The contribution of the pentose cycle to glucose metabolism was determined from the ratio of specific activity of Carbon 1 of the glucose unit of glycogen versus Carbon 2 as described by Wood et al.[19] using glucose-2-C-14 as substrate. A discussion of these methods as applied to islet has appeared earlier.[15]

RESULTS

Table 1 shows the distribution of C-14 in Carbons 1, 2 and 3 of the glucose unit of glycogen following incubation of islet with glucose-2-C-14. The pentose cycle contribution was calculated by the equation $H1/H2 = 2PC/1 + 2PC$, where H1 and H2 are the relative specific activities of Carbons 1 and 2 of the glucose unit of glycogen and PC is the fractional pentose cycle contribution to glucose metabolism as defined by Wood et al.[19] At the normal glucose concentration of the goosefish, 0.25 mg./ml., 10.8 per cent of the glucose was metabolized by the pentose cycle and glucagon did not alter this percentage. Tolbutamide caused a small but statistically significant reduction in pentose cycle contribution to 8.8 per cent while hyperglycemia (2.00 mg./ml.) reduced it to 4.3 per cent.

The production of C-14-O_2 from glucose-6-C-14 incubated with islet is shown in table 2. Slices incubated with glucagon and tolbutamide did not differ significantly from the 0.25 mg./ml. glucose control. Glucose

TABLE 2

Effect of glucagon, tolbutamide and hyperglycemia on C-14-O_2 production from glucose-6-C-14 by goosefish islet, in vitro

	Mean DPM/gm. islet/3 hr.
Glucose, 0.25 mg./ml.	139,000
Glucose + glucagon, 5 μg./ml.	166,000*
Glucose + tolbutamide 200 μg./ml.	127,000*
Glucose, 2.00 mg./ml.	645,000†

Glucose specific activity corrected to 1 μC./mg.
Statistics by analysis of variance, n = 11.
*$p > 0.5$ versus control.
†$p < 0.001$ versus control.

TABLE 1

Distribution of C-14 in the glucose unit of glycogen after incubation of goosefish islet with glucose-2-C-14

	Glucose, 0.25 mg./ml.				Glucose, 0.25 mg./ml. + tolbutamide, 200 μg./ml.			
Exp. #	1	2	3	4	1	2	3	4
Carbon 1	15.9	20.0	18.6	16.5	13.5	17.7	15.8	13.7
2	100	100	100	100	100	100	100	100
3	7.7	14.2	26.2	13.4	9.6	13.0	27.8	13.1
Recovery (per cent)	87	83	89	90	66	84	88	93
Per cent PC	9.5	12.5	11.4	9.9	7.8	10.2	9.4	7.5
Mean per cent PC	10.8				8.8	($p < 0.005$ versus control)*		
	Glucose, 0.25 mg./ml. + glucagon, 5 μg./ml.				Glucose, 2.00 mg./ml.			
Exp. #	1	2	3	4	1	2		
Carbon 1	14.5	18.6	18.5	18.2	7.6	8.1		
2	100	100	100	100	100	100		
3	10.7	12.6	31.1	28.3	6.5	6.9		
Recovery (per cent)	83	88	88	88	94	—		
Per cent PC	8.5	11.7	11.3	11.1	4.1	4.4		
Mean per cent PC	10.6	($p > 0.5$ versus control)*			4.3			

In the distributions shown above, Carbon 2 has been set equal to 100, and the other values expressed relative to Carbon 2.
*Statistics by analysis of variance. Since only two samples were degraded, the glucose, 2.00 mg./ml., values were not included in the statistical analysis.

at a concentration of 2.00 mg./ml. caused a 4.6-fold increase in the production of C-14-O_2.

The uptake of glucose by islet when the glucose concentration was 0.25 mg./ml. was 465 μg./gm. (table 3). Glucagon and tolbutamide caused small but not statistically significant increase in glucose uptake. Glucose at 2.00 mg./ml. caused a 6.5-fold increase in uptake to 3,050 μg./gm. islet/hr.

TABLE 3

Effect of glucagon, tolbutamide and hyperglycemia on glucose uptake by goosefish islet (μg./gm./hr.)

	Mean+1 S.E.M.
Glucose, 0.25 mg./ml.	465± 60 (11)
Glucose + glucagon, 5 μg./ml.	490±165 (12)*
Glucose + tolbutamide, 200 μg./ml.	585± 28 (11)*
Glucose, 2.00 mg./ml.	3,050±406 (13)†

Statistics by *t* test.
*p > 0.5 versus control.
†p < 0.001 versus control.

The specific yield of C-14-O_2 from glucose-6-C-14 is a measure of the contribution of the Krebs cycle to glucose oxidation. The values shown in table 4 are expressed as a percentage and were calculated according to Katz and Wood.[17] In the 0.25 mg./ml. glucose medium, 4.88 per cent of the glucose taken up was oxidized to CO_2 by the Krebs cycle; in the presence of glucagon and tolbutamide the values were 5.80 per cent and 4.56 per cent respectively. At 2.00 mg./ml. glucose, Krebs cycle oxidation was 3.87 per cent; none of the latter values were significantly different from the control.

DISCUSSION

The assumptions required in the calculation of the pentose cycle contribution to glucose metabolism in the goosefish islet have been discussed previously.[15] In the 0.25 mg./ml. and 2.00 mg./ml. glucose experiments using glucose-2-C-14 as substrate, the relative specific activity of Carbon 1 versus Carbon 2 of the glucose unit of glycogen was slightly greater than reported previously.[15] However, with hyperglycemia, PC decreased by 60 per cent which compares favorably with the 63 per cent decrease noted previously.[15]

TABLE 4

Effect of glucagon, tolbutamide and hyperglycemia on the per cent specific yield of C-14-O_2 from glucose-6-C-14 in goosefish islet, in vitro

Treatment	Mean+1 S.E.M.
Glucose, 0.25 mg./ml.	4.88±0.64
Glucose + glucagon, 5 μg./ml.	5.80±0.73*
Glucose + tolbutamide, 200 μg./ml.	4.56±0.54*
Glucose, 2.00 mg./ml.	3.87±0.85*

*p > 0.5 versus 0.25 mg./ml. glucose control by *t* test, n = 9.

In Experiment 3, there was considerable randomization of C-14 into Carbon 3 relative to Carbon 1. This was noted in an earlier study with goosefish islet[15] and is thought to be related to reversal of the transaldolase, transketolase series of reactions recently discussed in detail by Katz and Rognstad.[20] They indicated that reasonable estimates of the pentose cycle can frequently be made in spite of transaldolase-transketolase exchange if the ratio of C1 to C2 is used.[20] The production of lactate under these in vitro conditions was not measured but is probably considerable. No data are currently available on the pathways of glucose metabolism and lactate production by islet, in vitro.

Glucose uptake by goosefish islet at a glucose concentration of 2.00 mg./ml. was 3,050 μg./ml. This value is considerably higher than the value reported previously[15] and is probably due to a higher ratio of medium to tissue in these studies.

When the conversion of fructose-6-phosphate to fructose-1, 6-diphosphate is irreversible and the triose phosphates are in complete and rapid equilibrium, the percentage of the glucose-6-C-14 utilized which is converted to C-14-O_2 is a measure of the contribution of the Krebs cycle to glucose oxidation. When goosefish islet was incubated with glucose-6-C-14, the relative specific activity of Carbon 1 of the glucose unit of glycogen was 2.6 compared with 100 for Carbon 6, suggesting only slight reformation of glucose-6-phosphate from triose-phosphate.[15] Thus, it would appear justified in goosefish islet to regard the specific yield of C-14-O_2 from glucose-6-C-14 as an adequate approximation of the fractional contribution of the Krebs cycle to glucose oxidation. The values for Krebs cycle oxidation should be considered as maximum values, however, due to the presence of small amounts of C-14 appearing in Carbon 1 of the glucose-6-phosphate formed during the incubation which can be oxidized to C-14-O_2 by the pentose cycle.

Hyperglycemia (2.00 mg./ml. glucose) caused a decrease in the pentose cycle contribution to glucose metabolism from a control value of 10.8 per cent to 4.3 per cent, but did not affect the per cent of glucose carbon metabolized by the Krebs cycle. Glucagon did not alter the glucose metabolized by the pentose or

TABLE 5

Calculation of glucose metabolized by the pentose and Krebs cycles and estimation of production rate of NADH and NADPH in goosefish islet, in vitro

	Glucose uptake μg./gm./hr.	Per cent PC	Per cent KC	Glucose μg. ox. by PC /gm./hr.	Glucose μg. ox. by KC /gm./hr.	μM.* NADPH prod. /gm./hr.	μM.† NADH prod. /gm./hr.
Glucose, 0.25 mg./ml.	465	10.8	4.9	50.2	22.8	1.68	1.27
Glucose + tolbutamide, 200 μg./ml.	585	8.8	4.6	51.5	26.9	1.71	1.49
Glucose + glucagon, 5 μg./ml.	490	10.6	5.8	51.2	28.4	1.74	1.58
Glucose, 2.00 mg./ml.	3,050	4.3	3.9	131.0	119.0	4.38	6.67

*Example of NADPH calculation, glucose 0.25 mg./ml.: Over-all reaction = 1 glucose + 6 NADP → 6 CO_2 + triose-P ± 6 NADPH, ∴ (50.2/180) × 6 = 1.68 μM. NADPH/gm./hr.

†Example of NADH calculation, glucose 0.25 mg./ml. Over-all reaction = 1 glucose + 10 NAD → 6 CO_2 + 10 NADH, ∴ (22.8/180) × 10 = 1.27 μM. NADH/gm./hr.

Krebs cycles. Tolbutamide did not alter Krebs cycle glucose metabolism but caused an 18 per cent decrease in glucose metabolized by the pentose cycle. The significance of this small decrease is uncertain.

Calculations of net glucose metabolism were made to show the magnitude of the effects of hyperglycemia, and tolbutamide and glucagon on glucose oxidation by the pentose and Krebs cycles (table 5). Hyperglycemia caused an increase in glucose oxidized by the pentose cycle from 50.2 μg./gm./hr. to 131 μg./gm./hr. while metabolism of glucose via the Krebs cycle rose from 22.8 μg./gm./hr. to 119 μg./gm./hr. Tolbutamide and glucagon did not affect net glucose oxidation by either pathway. Since NADH, NADP, and NADPH have recently been reported to increase insulin release in fish islet, in vitro,[14] a calculation of the net rate of formation of reduced pyridine nucleotides in fish islet was made from the amount of glucose metabolized by the pentose and Krebs pathways (table 5). Hyperglycemia caused an increase in the estimated rate of formation of reduced pyridine nucleotides in fish islet while glucagon and tolbutamide values were nearly identical with that for the 0.25 mg./ml. glucose control. These results are consistent with the concept that glucagon and tolbutamide exert their effects on insulin release at a site other than glucose metabolic pathways.

The glycogen content of goosefish islet is considerable (0.27 per cent wet weight),[21] and if a large fraction of the glycogen in islet were converted to glucose-6-phosphate during the incubation, significant increases in net pentose cycle or Krebs cycle oxidation could occur. The action of glucagon to promote insulin release in the absence of glucose could be explained by glycogenolysis in islet, as suggested by Devrim and Recant.[7] However, in the experiments using glucose-2-C-14, the incorporation of C-14 into glycogen was measured. Tolbutamide and glucagon did not significantly affect this incorporation when compared with the glucose control (0.25 mg./ml.). This finding is consistent with the data of Hellman and Idahl who reported that glucagon (5 μg./ml.) caused only small decreases in the glycogen content of microdissected islets from obese hyperglycemic mice.[22]

The net increase in glucose oxidation and pyridine nucleotide generation by the pentose and Krebs cycles caused by hyperglycemia may be related to insulin release by islet. However, the procedures used for measuring pentose cycle and Krebs cycle reflect metabolic events over the two-hour incubation period, and do not indicate metabolic events in the early minutes of exposure of the islet to glucose, glucagon and tolbutamide when insulin release might be greatest. In addition, the results indicate metabolic events in all islet cell types (A,B,C, and D cells) and do not indicate metabolism in the B cell. The results are not inconsistent with the hypothesis that glucose metabolism by the pentose cycle is related to insulin release, since hyperglycemia could increase the B cell pentose cycle while decreasing it in other islet cell types.

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REFERENCES

[1] Malaisse, W. J., Lea, M. A., and Malaisse-Lagae, F.: The effect of mannoheptulose on the phosphorylation of glucose and the secretion of insulin by islets of Langerhans. Metabolism *17*:126-32, 1968.

[2] Coore, H. G., Randle, P. G., Simon, E., Kracier, P. F., and Shelesnyak, M. C.: Block of insulin secretion from the pancreas by D-mannoheptulose. Nature *197*:1264-66, 1963.

[3] Lacy, P. E., Young, D. A., and Fink, C. J.: Studies on insulin secretion in vitro from isolated islets of the rat pancreas. Endocrinology *83*:1155-61, 1968.

[4] Coore, H. G., and Randle, P. J.: Regulation of insulin secretion studied with pieces of rabbit pancreas incubated in vitro. Biochem. J. *93*:66-78, 1964.

[5] Grodsky, G. M., Bennett, L. L., Smith, D. F., and Schmid, F. G.: Effect of pulse administration of glucose or glucagon on insulin secretion in vitro. Metabolism *18*:222-33, 1967.

[6] Grodsky, G. M., and Forsham, P. H.: Insulin and the pancreas. Ann. Rev. Physiol. *28*:347-80, 1966.

[7] Devrim, S., and Recant, L.: Effect of glucagon on insulin release, in vitro. Lancet *2*:1227-32, 1960.

[8] Montague, W., and Taylor, K. W.: Penitols and insulin release by isolated rat islets of Langerhans. Biochem. J. *109*: 333-39, 1968.

[9] Montague, W., and Taylor, K. W.: Insulin secretion and the intracellular concentrations of glucose-6-phosphate and 6-phosphogluconate in isolated islets of Langerhans. Biochem. J. *109*:30P-31P, 1968.

[10] Bauer, G. E., Lindall, A. W., Jr., Dixit, P. K., Lester, G., and Lazarow, A.: Subcellular distribution of leucine-H radioactivity during incubation of goosefish islet tissue. J. Cell. Biol. *28*:413-21, 1966.

[11] Sorenson, R. L., Lindall, A. W., and Lazarow, A.: Studies on the isolated goosefish insulin secretion granule. Diabetes *18*:129-37, 1969.

[12] Watkins, D., Leonards, J., Dixit, P. K., Cooperstein, S. J., and Lazarow, A.: Glucose stimulation of insulin release from toadfish islet tissue, in vitro. Biol. Bull. *127*:395-96, 1964.

[13] Watkins, D., and Cooperstein, S. J.: Insulin secretion from toadfish islet tissue stimulated by pyridine nucleotides. Science *162*:283-84, 1968.

[14] Watkins, D.: Personal communication.

[15] Hostetler, K. Y., Cooperstein, S. J., Landau, B. R., and Lazarow, A.: Pathways of glucose metabolism in the isolated islet of the goosefish, in vitro. Amer. J. Physiol. *211*:1057-62, 1966.

[16] Hostetler, K. Y., and Landau, B. R.: Estimation of the pentose cycle contribution to glucose metabolism in tissue, in vivo. Biochemistry *6*:2961-64, 1967.

[17] Katz, J., and Wood, H. G.: The use of $C^{14}O_2$ yields from glucose-1- and -6-C^{14} for the evaluation of the pathways of glucose metabolism. J. Biol. Chem. *238*:517-23, 1963.

[18] Rognstad, R., and Katz, J.: The balance of pyridine nucleotides and ATP in adipose tissue. Proc. Nat. Acad. Sci. USA *55*:1148-56, 1966.

[19] Wood, H. G., Katz, J., and Landau, B. R.: Estimation of pathways of carbohydrate metabolism. Biochem. Z. *338*:809-47, 1963.

[20] Katz, J., and Rognstad, R.: The labeling of pentose phosphate from glucose-C^{14} and estimation of the rates of transaldolase, transketolase, and the contribution of the pentose cycle, and ribose phosphate synthesis. Biochemistry *6*:2227-46, 1967.

[21] Lazarow, A.: Functional characterization and metabolic pathways of the pancreatic islet tissue. Recent Progr. Hormone Res. *19*:489-546, 1963.

[22] Hellman, B., and Idahl, L.: Presence and mobilization of glycogen in mammalian pancreatic B-cells. Endocrinology *84*: 1-8, 1969.