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Effects of Glucose on Glucagon Secretion by the Anolian Splenic Pancrease (40016)

WILLIAM B. RHOTEN

Department of Physiology and Biophysics and Program in Anatomical Sciences, School of Basic Medical Sciences, University of Illinois, Urbana, Illinois 61801

Introduction. The clinical hallmark of human diabetes mellitus is hyperglycemia. Hyperglycemia is a distinctive characteristic of the American anole, *Anolis carolinensis* (1). An attenuated insulin secretory response to glucose is observed in the anole (2, 3), unborn mammals including man (4–7), and prediabetics and diabetics (8–10). Recently we presented evidence that the anole has fasting hyperglucagonemia relative to the prevailing level of blood glucose (11). The significance of glucagon in human diabetes is not established. Results which suggest that glucagon may be important in the mediation of diabetic hyperglycemia and the clinical course of the disease have been reported (12–14). Some morphological evidence has demonstrated a relative or absolute increase in the number of α cells in juvenile diabetes (15, 16). Approximately 50% of the endocrine cells in the anolian pancrease are α cells (1). Pancreatectomy in the α cell-rich lizards results in a rapid onset of hypoglycemia which persists for several days (17, 18). The present investigation was initiated in order to better characterize α cell regulation in this species.

Materials and methods. Experimental procedures. Two days prior to sacrifice, adult anoles were placed in an environmental chamber with 14 hr of light alternating with 10 hr of darkness. The temperature was 33° during the light cycle and 23° during the dark cycle. The splenic portion of the pancreas was removed from decapitated lizards. Each splenic pancreas was placed in Hanks' buffer, pH 7.3, and freed of extraneous tissue. The splenic pancreata were initially incubated for 30 min at 33 ± 0.5° in disposable cups for automatic analysis (W. Sarstedt, Inc., Princeton, N.J.) in 0.3 ml of incubation medium. The basic incubation medium was a Krebs–Ringer–bicarbonate solution containing 2.25 mg/ml of D-glucose (National Bureau of Standards, Washington, D.C.), 0.5% (w/v) plasma albumin (type IV, Sigma Chemical Co., St. Louis, Mo.), 500 kIU of aprotinin/0.3 ml (Sigma Chemical Co.), and, in milliequivalents per liter: Na, 141.5; chloride, 129.9; Ca, 5.8; bicarbonate, 20.0; phosphate, 5.0; K, 5.0; and Mg, 2.24.

The cups, each containing two splenic pancreata, were placed in glass scintillation vials and capped with serum bottle stoppers. The vials were placed in a reciprocating shaker bath (100 strokes/min, 4-cm amplitude) and continuously gassed with a humidified mixture of 95% O_2–5% CO_2 to maintain pH 7.3–7.4. After the equilibration period the medium was removed by aspiration and 0.3 ml of the basic buffer was added (basal incubation). After 30 min, the medium was aspirated off and medium containing various concentrations of D-glucose and, in some cases, arginine (L-Arg HCl) was added (experimental incubation). Incubation was continued for 60 min. The medium was aspirated off and the pancreata were blotted and weighed. For dynamic studies 8 or 10 splenic pancreata were perifused as described previously (2).

Radioimmunoassay of glucagon. Conditions and characteristics of the glucagon radioimmunoassay have been described previously (11). Glucagon levels were determined with incubation medium at the appropriate dilutions (1:20 to 1:200). All samples were assayed in duplicate at two dilutions which resulted in less than 50% displacement of tracer hormone. Standard curves were generated from medium at corresponding dilutions. The porcine glucagon standard was generously provided

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by Dr. Mary Root of the Eli Lilly Research Laboratories. The radiolabeled hormone, [^{125}I]glucagon, was diluted with assay buffer to yield a standard curve of suitable displacement (New England Nuclear, Cambridge, Mass., or Cambridge Nuclear, Billerica, Mass.). Glucagon antisera were kindly provided by Drs. Anthony S. Pagliara and Howard S. Tager. The characteristics of Dr. Tager's antisera have been described (19).

Results. The mean glucagon release during the basal incubation (2.25 mg/ml of glucose) for all experiments was 31.6 ± 5.1 pg/mg of splenic pancreas/min (N = 37). The range of secretion for all basal incubations was 2.1 to 141.5 pg/mg/min. Glucagon release during the basal incubation was not significantly different from the release with the same concentration of glucose during the experimental incubation of 60 min ($P > 0.05$ by unpaired t test). This result was observed in spite of the more narrow range of values for glucagon secretion during the experimental period (2.3 to 47.6 pg/mg/min).

A 56% decrease in the concentration of glucose (1.0 mg/ml) failed to evoke a significant change in the rate of glucagon release (Table I). The addition of arginine (10 mM) at the same concentration of glucose did not significantly alter the rate of release. A significant increase in glucagon release did occur when the glucose concentration was decreased to 0.5 mg/ml (Table I). In the presence of arginine, glucagon release was not significantly different from that obtained in its absence ($P > 0.4$ by unpaired t test).

The kinetics of glucagon secretion when splenic pancreata were perifused with the combination of 0.5 mg/ml of glucose and arginine (10 mM) are shown in Fig. 1. A striking increase in glucagon release occurred rapidly after changing to this medium. Enhanced release developed concomitantly with the fall in glucose concentration below 1.0 mg/ml. The peak of early release occurred between 4 and 8 min after the glucose level fell below 1.0 mg/ml. The mean maximal rate of secretion during the first 8 min was 1.02 ± 0.17 ng/mg of splenic pancreas/min.

A high concentration of glucose, 6 mg/ml, failed to significantly decrease gluca-

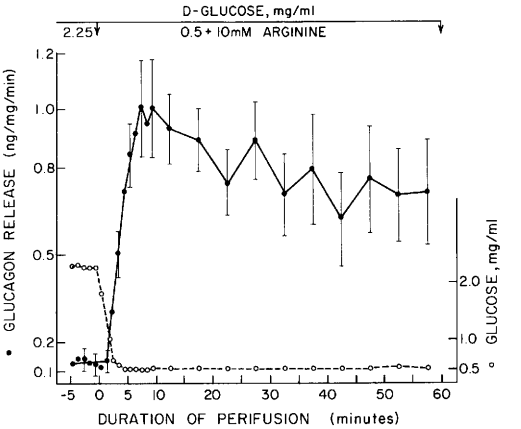

FIG. 1. Pattern of glucagon release from splenic pancreata perifused with 0.5 mg/ml of glucose and 10 mM arginine. The splenic pancreata were initially perifused with 2.25 mg/ml of glucose for 60 min. The medium was changed to one containing low glucose and arginine, and perifusion was continued for another 60 min. Time 0 is 2 min after changing to the new medium. Vertical bars represent the standard errors of the means. The number of observations was three.

TABLE I. GLUCAGON RELEASE FROM ANOLIAN SPLENIC PANCREATA INCUBATED WITH VARIOUS CONCENTRATIONS OF GLUCOSE.[a]

Basal incubation (2.25 mg/ml of glucose)	Experimental conditions		Number of observations	Statistical analysis
	Glucose (mg/ml)	Glucagon release		
32.0 ± 12.8	2.25	32.2 ± 8.3	6	NS
27.3 ± 4.9	1.0	49.2 ± 7.0	4	NS
29.2 ± 7.3	1.0 + 10 mM Arg	113.1 ± 37.7	6	NS
36.0 ± 20.4	0.5	217.9 ± 59.0	7	<0.005
20.2 ± 5.9	0.5 + 10 mM Arg	299.3 ± 90.3	7	<0.02
43.0 ± 13.0	6.0	15.4 ± 3.7	7	NS

[a] Results are expressed as picograms of porcine glucagon equivalents per milligram of splenic pancreas per minute ± SEM. Glucagon release under experimental conditions was compared with release during the basal incubation by means of the paired t test. A P value less than 0.05 was considered significant.

gon secretion (Table I). The range of release was 5.1 to 28.0 pg/mg of splenic pancreas/min.

Discussion. This study has shown that glucagon secretion by the anolian splenic pancreas is relatively insensitive to changes in the concentration of glucose. *Anolis carolinensis* has a normal fasting blood glucose level of about 2.2 mg/ml (1, 2, 20). In either relative or absolute terms, glucagon release in response to a low concentration of glucose is attenuated in the anolian α cell. Arginine does not correct this deficit. The anolian α cell responds rapidly when the level of glucose is severely depressed. This rapid onset of glucagon secretion is similar to that found in either perfused rat pancreas (21) or perifused isolated rat islets (22).

Studies with other experimental animals have shown that metabolic substrates other than glucose may have significant physiologic regulatory roles in islet cell function (23–25). The absence of a significant effect of arginine on glucagon release by the anole is in marked contrast to mammals, including man, where numerous studies have shown an increase in glucagon secretion in response to arginine. Prior studies have shown that glucose, arginine, and leucine are effective stimuli of insulin release from the anolian β cell (2, 3, 26). However, the influence of other substrates on α and β cell function is virtually unknown for these animals. Similarly, there is a paucity of information regarding substrate utilization by peripheral tissues in lizards. Nevertheless, some studies provide suggestive evidence of a major role for lipid metabolism in lizards (1, 27–30). Therefore, it seems realistic to consider the possibility that lipids and/or protein may have a preeminent role in saurian homeostasis and that metabolic substrates other than glucose may have a substantial regulatory role in islet cell function in these animals.

Summary. Glucagon secretion from splenic pancreata of *Anolis carolinensis* was not significantly increased by a 56% decrease in the concentration of glucose (1 mg/ml). When the glucose concentration was reduced to 0.5 mg/ml, a significant increase in glucagon release was observed. Arginine (10 m*M*) did not significantly alter glucagon secretion. The pattern of glucagon secretion under stimulatory conditions shows a rapid onset and long duration. A concentration of glucose of 6 mg/ml failed to significantly decrease glucagon release. The results demonstrate the utility of the anolian splenic pancreas for exploring the regulation and dynamics of glucagon secretion.

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