GLUCAGON LEVELS IN PANCREATIC EXTRACTS AND PLASMA OF THE LIZARD (1)

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ABSTRACT Levels of glucagon in the splenic pancreas and in plasma of the lizard Anolis carolinensis were estimated by radioimmunoassay. The splenic pancreas of Anolis has a glucagon concentration nearly a 1000 times greater, on a weight basis, than that of the mammalian pancreas. Glucagon-like immunoreactivity (GLI) of anolian plasma varied over a wide range, but relative to mammals the GLI levels were inappropriately elevated for the concentration of plasma glucose. The anole may prove valuable in studies on the regulation of glucagon secretion, particularly as related to alpha cell function in the diabetic state.

Human diabetes mellitus is accompanied by inappropriately elevated levels of glucagon relative to the concentration of blood glucose (Unger and Lefebvre, '72; Buchanan and McCarroll, '72; Gerich et al., '75). In the practice of medicine, hyperglycemia is the sine qua non for the diagnosis of diabetes. The beta cells of prediabetics and diabetics are characterized by low insulin secretion in response to glucose (Cerasi, '75; Cerasi and Luft, '67; Fujita et al., '75). Normal, adult American anoles (Anolis carolinensis) are markedly hyperglycemic in comparison to mammals and the anolian beta cell is relatively insensitive to glucose (Rhoten, '73, '74). The present findings suggest that the anole, like the human diabetic, exhibits hyperglucagonemia relative to the concentration of blood glucose.

MATERIALS AND METHODS To determine levels of glucose and glucagon in plasma, tail blood (0.1 - 0.2 ml) from adult anoles was collected in heparinized capillary tubes and dispensed into chilled, fluoride-coated 250-µl tubes

(Beckman Instrument) containing 87.3 μg benzamidine HCl monohydrate and 100 μg EDTA (disodium salt). After centrifugation, 20 μl of plasma was removed for measurement of glucose levels (Glucostat Ultramicro, Worthington Biochem.). The plasma for the glucagon assay was diluted 1:10 or 1:20 with assay buffer of the following composition: 0.05 M Na-K phosphate, 33.4 mM NaCl, 5 mM benzamidine HCl, 0.5 mM merthiolate and 5 mg/ml of bovine albumin, final pH 7.40. The diluted plasma samples were frozen in dry ice and acetone and stored at -20° C. For measurement of pancreatic glucagon, individual splenic pancreata were removed, blotted, weighed and homogenized in acidic ethanol containing 5 mM benzamidine HCl. The homogenate was extracted for 24 hr. After centrifugation, the extract was decanted and aliquots were diluted 1:100 with assay buffer and frozen at -20° C.

Anti-glucagon serum (lot #4, B-9) was the generous gift of Doctor Anthony S. Pagliara. Porcine glucagon was kindly provided by Doctor Mary Root of the Lilly Research Laboratories. Glucagon radioimmunoassay was accomplished by a modification of the ethanol separation procedure of Heding ('71). Labeled glucagon (^{125}I) was purchased from Cambridge Nuclear. Antiserum, ^{125}I-glucagon, standards and samples were made up in the assay buffer. Radioimmunoassay was carried out utilizing the following procedure. Using duplicates or triplicates, 200 μl of standard (containing 12.5 to 200 pg of glucagon) or sample received 100 μl of diluted antiserum. After addition of antiserum and subsequent incubation at 4° C for 24 hr, 100 μl of ^{125}I-glucagon (50 pg) was added and the tubes were incubated for another 24 hr at 4° C. Under these conditions a final antiserum dilution of 1:12000 bound approximately 67% of the labeled glucagon. Calculation of the results and additional assay controls were similar to those used in the "back-titration" assay of insulin (Makulu et al., '69; Doctor Peter H. Wright, personal communication).

RESULTS The diluted extracts of the anolian splenic pancreas exhibited good parallelism with standard (fig. 1). Recovery of glucagon standard following extraction was about 92%. The smallest amount of glucagon standard assayed under the present conditions was 6.25 ng. This amount of glucagon significantly altered the % binding of labeled glucagon (calculated from five individual standard curves: $P<0.01$ by t test, d.f. = 3). Interassay precision was satisfactory as estimated by the standard error of the mean (SEM) (fig. 1). The mean immunoreactive glucagon level for seven anolian splenic pancreata was 3.39 µg porcine equivalents/mg wet weight. The range of values was 2.39 to 4.98 µg equivalents/mg with a SEM of 0.34. The glucagon content of the splenic pancreas for each lizard was estimated by averaging the porcine equivalents obtained at four dilutions of the extract (fig. 1).

For comparison with plasma glucagon levels in Anolis, rat plasma and plasma from a single human subject were assayed. GLI levels of plasma samples from the rats and the human subject were high (fig. 2). This result can be attributed in part to the nonspecific reactivity of the antiserum for pancreatic glucagon (Doctor Anthony Pagliara, personal communication). Measurement of GLI in human plasma was further complicated by the nonproportional results obtained with dilution (fig. 3). For the rat there was only a slight loss of linearity with dilution (fig. 3). Since anolian plasma was assayed at a dilution of 1:10 or 1:20, nonspecific effects were likely to be minimal (Yalow and Berson, '71).

Although plasma GLI varied widely in Anolis, the values tended to be considerably higher than those obtained for mammals using the identical assay procedure (fig. 2). Even more surprising was the finding of these high levels of plasma GLI in association with elevated plasma glucose (fig. 2).

DISCUSSION The anolian splenic pancreas contains, on a weight basis, several hundred times as much glucagon as mammals. Assan et al. ('69) reported

1.5 μg of glucagon/g of pancreas for man and 1.2 μg/g for the rat. Unger ('72), using a specimen of human pancreas obtained at biopsy, found 9 μg/g. Two specimens of human pancreas obtained at autopsy, extracted and partially purified by the method of Davoren ('62) and assayed according to the described procedure, contained 7.7 and 6.6 μg glucagon/g (Rhoten, unpublished observations). The predominance of alpha cells (fig. 4) and the large concentration of glucagon in the anolian splenic pancreas indicate that this species may be a useful model for exploring the regulation of glucagon secretion and the molecular interaction underlying α cell function.

Since all vertebrates probably have gastrointestinal GLI (Assan et al., '69; Östberg et al., '76) it is likely that the plasma values reported here include such activity. The value obtained for the human subject is well within the range reported by other authors using a nonspecific antiserum (Luyckx, '72). In any case, the values obtained in Anolis are not only higher than those found in normal mammals, but occur concomitantly with elevated levels

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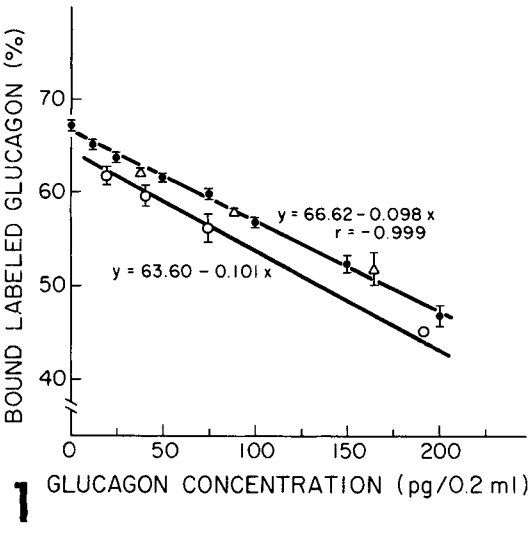
FIGURE LEGENDS

1 Dose-response relationships obtained in the glucagon assay. The reference preparation was porcine glucagon (● , n = 8). Extracts of a splenic pancreas of Anolis (○) and a human pancreas (△) were diluted with assay buffer. Values are expressed as porcine glucagon equivalents. Vertical bars represent the standard errors of the means (SEM).

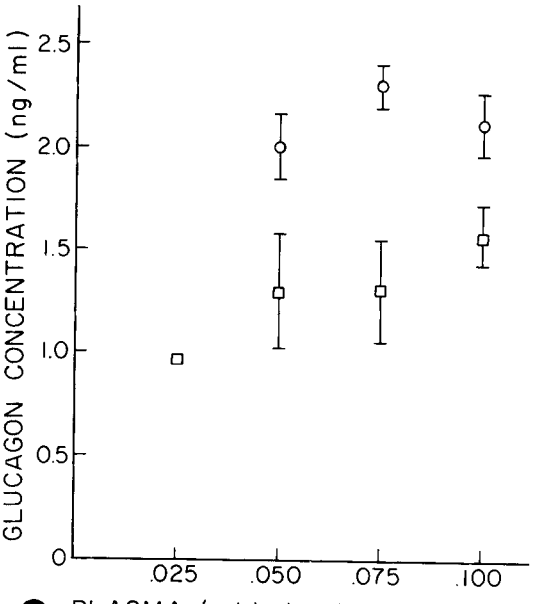
2 Relationships between plasma glucose levels and plasma glucagon-like immunoreactivity (GLI). GLI values for the anole (● , n = 17) were determined at a plasma dilution of 1:10. GLI values for the rat (○ , n = 4) and the human subject (△) were obtained from the results at three different dilutions of plasma.

3 Effect of plasma dilution on the apparent level of plasma GLI. Values (rat, ○ ; human, □) are corrected for dilution and are expressed as porcine equivalents. Vertical bars indicate SEM.

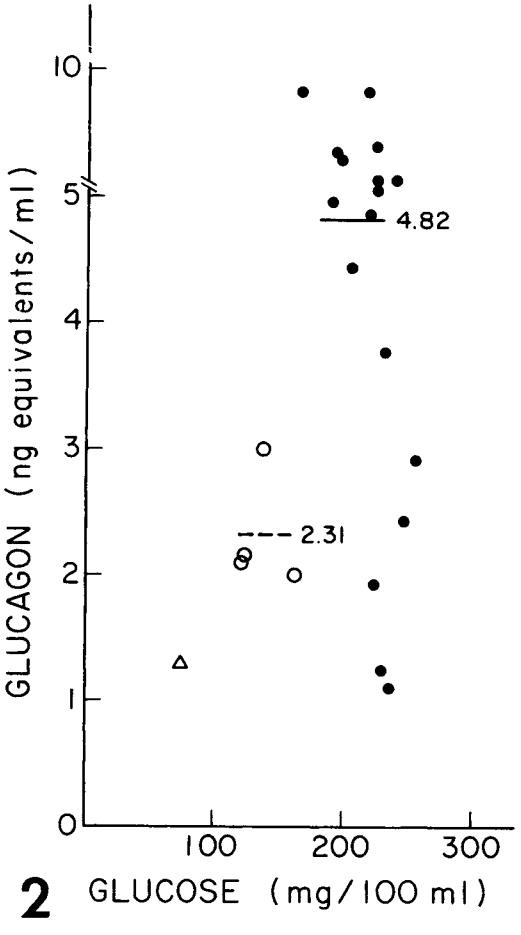
4 Darkfield micrograph of epoxy section (about 1 μm) of splenic pancreas of Anolis (fixed in 3% glutaraldehyde-2% formaldehyde and processed for electron microscopy) illustrating alpha (A), beta (B), and delta (D) cells. X 960.


BOUND LABELED GLUCAGON (%)
70
60
50
40
0
50
100
150
200
y = 66.62 - 0.098 x
r = -0.999
y = 63.60 - 0.101 x
GLUCAGON CONCENTRATION (pg/0.2 ml)

1


GLUCAGON CONCENTRATION (ng/ml)
2.5
2.0
1.5
1.0
0.5
0
.025
.050
.075
.100
PLASMA (ml in incubation mixture)

3


GLUCAGON (ng equivalents/ml)
10
5
4
3
2
1
0
4.82
2.31
100
200
300
GLUCOSE (mg/100 ml)

2


A
B
D

4

of blood glucose. These data suggest that in Anolis secretion of glucagon is maintained in spite of elevated blood glucose levels. Such levels of blood glucose significantly suppress glucagon release in normal mammals. Experiments are in progress to examine glucose regulation of anolian α cell secretion. If hyperglycemia does not significantly reduce glucagon secretion, then another parallelism between the diabetic state in man and the anolian model will have been identified.

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LITERATURE CITED

Assan, R., G. Tchobroutsky and G. Rosselin 1969 Caracterisation radioimmunologique de glucagon dans les tissus digestifs de diverses especes animales. Path. Biol., 17: 747-755.

Buchanan, K. D., and A. M. McCarroll 1972 Abnormalities of glucagon metabolism in untreated diabetes mellitus. Lancet, II: 1394-1395.

Cerasi, E. 1975 Mechanisms of glucose stimulated insulin secretion in health and in diabetes: some re-evaluations and proposals. Diabetologia, 11: 1-13.

Cerasi, E., and R. Luft 1967 Insulin response to glucose infusion in diabetic and nondiabetic monozygotic twin pairs. Acta Endocrinol., 55: 330-345.

Davoren, P. R. 1962 The isolation of insulin from a single cat pancreas. Biochim. Biophys. Acta, 63: 150-153.

Fujita, Y., A. L. Herron and H. S. Seltzer 1975 Confirmation of impaired early insulin response to glycemic stimulus in nonobese mild diabetics. Diabetes, 24: 17-27.

Gerich, J. E., M. Lorenzi, D. M. Bier, V. Schneider, E. Tsalikian, J. H. Karam and P. H. Forsham 1975 Prevention of human diabetic ketoacidosis by somatostatin. New Engl. J. Med., 292: 985-989.

Heding, L. G. 1971 Radioimmunological determination of pancreatic and gut glucagon in plasma. Diabetologia, 7: 10-19.

Luyckx, A. S. 1972 Immunoassays for glucagon. In: Glucagon. P. J. Lefebvre and R. H. Unger, eds. Pergamon Press, Inc., New York, pp. 285-298.

Makulu, D. R., D. Vichick, P. H. Wright, K. E. Sussman and P.-L. Yu 1969 Insulin immunoassay by back-titration using alcohol precipitation of insulin-antibody complexes. Diabetes, 18: 660-669.

Östberg, Y., S. Van Noorden, A. G. E. Pearse and N. W. Thomas 1976 Cytochemical, immunofluorescence, and ultrastructural investigations on polypeptide hormone containing cells in the intestinal mucosa of a cyclostome, Myxine glutinosa. Gen. Comp. Endocrinol., 28: 213-227.

Rhoten, W. B. 1973 Perifusion of saurian pancreatic islets and biphasic insulin release following high glucose. Comp. Biochem. Physiol., 45A: 1001-1007.
Rhoten, W. B. 1974 The sensitivity of saurian pancreatic islets to glucose. Amer. J. Physiol., 227: 993-997.
Unger, R. H. 1972 Glucagon and glucagon immunoreactivity in plasma and pancreatic tissues. In: Glucagon. P. J. Lefebvre and R. H. Unger, eds. Pergamon Press, Inc., New York, pp. 205-211.
Unger, R. H., and P. J. Lefebvre 1972 Glucagon physiology. Ibid, pp. 213-243.
Yalow, R. S., and S. A. Berson 1971 Problems of validation of radioimmunoassays. In: Principles of Competitive Protein-binding Assays. W. D. Odell and W. H. Daughaday, eds. J. B. Lippincott Co., Philadelphia, pp. 374-400.

REFERENCES

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