Vol. 89, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 13, 1979 Pages 907-912

ALTERED REGULATION OF PANCREATIC GLUCAGON IN MALE RATS DURING AGING[1]

Thomas L. Klug, Colette Freeman[2], Karen Karoly, and Richard C. Adelman[3]

Temple University Institute on Aging,
Fels Research Institute, and
Division of Biomedical Research, Philadelphia Geriatric Center
Philadelphia, Pennsylvania 19140

Received June 28,1979

SUMMARY: Immunoreactive pancreatic glucagon levels in portal vein blood following intragastric glucose administration were determined in fasted male Sprague-Dawley rats of 2-, 12-, and 24-months of age. Immunoreactive glucagon levels decline to less than one-half the fasting values in 2-month old rats following glucose administration, whereas immunoreactive glucagon levels increase two to three times the fasting values in 12- and 24-month old rats under the same conditions. Glucagon inhibits glucose-stimulated hepatic glucokinase adaptation and may interfere with insulin action in the regulation of hepatic glucose balance. Therefore, differences in the availability of glucagon may contribute significantly to delayed hepatic glucokinase adaptation in rats during aging.

Several studies have documented a progressive deterioration of glucose metabolism in the rat during aging (1-6). While alterations in glucose tolerance (4-6) and hepatic glucokinase adaptation (2,4) during aging are generally ascribed to changes in the insulin binding capacity of target tissues (4-6), such age-dependent alterations in glucose metabolism might also be due to increased levels of, or increased sensitivity to, an antagonist of insulin action - such as glucagon (7,8). Glucagon, a polypeptide of pancreatic A-cell origin, exerts its effects on carbohydrate metabolism primarily in the liver where it is involved in maintaining glucose homeostasis through its effects on hepatic glucose uptake and hepatic enzymes regulating glycogen synthesis, glycogenolysis, and gluconeogenesis (8,9). The purpose of the present article is: 1) to determine the effect of glucose administration on

[1] Supported in part by Research Grants AG-00368 and CA-12227 from NIH, and an Established Investigatorship of the American Heart Assoc. to R.C.A..
[2] Present address: NIAMD, NIH, Bethesda, MD 20014.
[3] To whom inquiries should be addressed at the Temple University Institute on Aging, 3400 N. Broad Street, Philadelphia, PA 19140.

0006-291X/79/150907-06$01.00/0

circulating glucagon levels in fasted male rats; and 2) to assess the effect of glucagon on glucose-stimulated hepatic glucokinase induction. The results imply that altered glucagon regulation contributes to age-dependent changes in hepatic glucokinase adaptation.

EXPERIMENTAL

Animals: Male, Sprague-Dawley rats were obtained at 2-, 12-, and 24-months of age from a colony maintained for R. C. Adelman at the Charles River Breeding Laboratories. These rats are cesarian-derived and maintained behind a pathogen-defined barrier under rigorously controlled conditions (10). Mean life-span of these rats is approximately 30-months, their maximal life-span is approximately 40-months, and at about 24-months of age they are virtually free of detectable gross pathology (10).

Materials: Guinea pig anti-glucagon serum (30K) was obtained from Dr. R. H. Unger; Trasylol from FBA Pharmaceuticals, New York; {^{125}I}-glucagon from Nuclear Medical Laboratories, Dallas; crystalline beef glucagon from Calbiochem; and sodium salts of ATP and NADP and a suspension of glucose 6-phosphate dehydrogenase from Boehringer-Mannheim Corp..

Treatments: Rats were fed ad libitum a pasteurized, sterilized Charles River Chow which is reported to be of constant per cent composition and component source. Periods of fasting were begun between 0800 and 1000 hours. All fasted rats were provided with drinking water ad libitum. Glucose was administered intragastrically with a feeding needle at 0800 - 1000 hours and at a dosage of 2.5 mmoles in 1.25 ml of water per 100 g. of body weight. Glucagon was administered by intraperitoneal injection immediately before glucose administration. Serum was collected from portal vein blood with the addition of Trasylol (100 KIU/ml) following brief ether anesthetization of the rat.

Assays: The concentration of pancreatic glucagon was determined by radioimmunoassay with an antiserum specific for pancreatic glucagon (11), with purified beef glucagon as standard. Hepatic glucokinase was measured by the procedure of Sharma and coworkers (12). Significance of differences in glucagon and hepatic glucokinase responses to glucose administration was evaluated by Student's *t*-test.

RESULTS

Changes in the concentration of immunoreactive glucagon (IRG) in serum collected from portal vein blood following intragastric administration of glucose to 3-day fasted male rats of the indicated ages are illustrated in Figure 1.. At 2-months of age, the concentration of IRG decreases significantly ($p < 0.05$), from 0.85 to 0.35 ng per ml of serum within 30 minutes and remains depressed throughout the duration of the experiment. At 12-months of age, the concentration of IRG increases significantly ($p<0.05$), from 0.45 to 0.85 ng per ml of serum over a 3-hour period, and then decreases to 0.40 ng per ml by 5-hours. At 24-months of age, the concentration of IRG increases significantly ($p<0.05$), from 0.45 to 1.40 ng per ml of serum within a 2-hour period, and

then decreases to approximately 0.70 ng per ml by 5 hours. Control experiments, in which 3-day fasted rats of the respective ages are administered water containing no glucose, indicate negligible effects of handling on the concentration of IRG in serum, although data are not presented.

The effect of glucagon treatment on hepatic glucokinase response to glucose administration in 2-month old rats is shown in Table 1.. In rats not receiving glucagon, glucokinase activity increases from a fasting value of 0.70 to 1.80 i.u. per g. of liver by 8 hours following glucose administration. Treatment of rats with 5 μg of glucagon immediately before glucose feeding, however, results in complete inhibition of glucokinase adaptation for several hours, and significant ($p<0.05$) depression of enzyme induction for a least 4 hours. A larger dose of 70 μg per rat results in inhibition of enzyme adaptation in response to glucose for at least 4 hours, and depression of glucokinase activity for perhaps as long as 8 hours.

DISCUSSION

The onset of hyperglycemia in normal man is followed by a rapid decline in serum IRG levels (13), a response very similar to that observed in fasted 2-month old rats following oral glucose administration (Figure 1.). In sharp contrast, serum IRG levels actually increase in response to hyperglycemia in the older rats, most significantly in the 24-month old rats (Figure 1.). The glucagon response of the older rats is similar to that reported to occur in human adult-onset diabetics following carbohydrate ingestion (13). The role of glucagon *in vivo* in the rat, however, may not be completely analogous to that in man as, for example, the importance of glucagon in blood glucose homeostasis in fasting rats is still uncertain (14,15).

The antagonistic interaction of insulin and glucagon, particularly as it relates to hepatic cyclic adenylate (cAMP) production and glucose balance, was demonstrated in perfused rat liver (16). In this system, relative glucagon excess elevated cAMP levels and resulted in increased net glucose output (16).

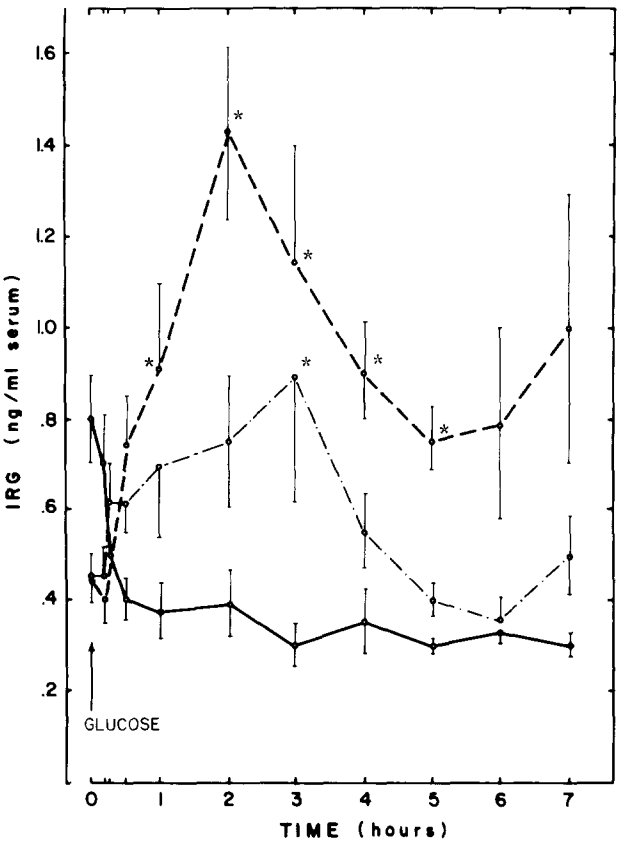

Figure 1. Effects of intragastric glucose administration on immunoreactive pancreatic glucagon (IRG) levels in portal vein serum of 2- (———), 12- (—·—·—·), and 24-(------) month old male Sprague-Dawley rats. (*) indicate IRG levels significantly greater than in 2-month old rats ($p<0.05$).

Recent studies indicate that, unlike insulin (4), glucagon binding (17) and glucagon-stimulated adenylate cyclase activity (18) are unimpaired in rat hepatocytes between 2- and 24-months of age. Therefore, significantly higher levels of glucagon in the portal vein blood of 12- and 24-month old rats following glucose administration may be partly responsible for altered glucose tolerance by their effects on net glucose uptake and/or output. Age-related increases in the magnitude of glucose-stimulated insulin secretion (3,4) may also be due to the insulinotropic activity of higher serum glucagon levels in the older rats (8).

The present findings may contribute to our understanding of the source of the altered capability for hepatic enzyme adaptation in the aging rat. The

Table 1. Effect of Glucagon on Hepatic Glucokinase Adaptation.

Glucagon Dose (µg)	Time Following Glucose Administration: Glucokinase Activity (i.u./g. liver)			
	0 HR	2 HR	4 HR	8 HR
0	0.70±0.05 (29)[+]	1.25±0.12 (13)	1.65±0.07 (33)	1.78±0.09 (17)
5	0.82±0.08 (5)	0.83±0.11[*] (5)	1.15±0.18[*] (6)	1.58±0.12 (3)
70	-----	-----	0.83±0.08[*] (6)	1.35±0.23 (6)

Fasted 2-month old rats were administered glucagon intraperitoneally immediately before intragastric glucose administration. Glucokinase activity was determined at the indicated times by the method given in the Experimental section.

[+]Number in parenthesis indicates the number of rats per group.
[*]Significantly less than response of rats not receiving glucagon ($p<0.05$).

time required to initiate glucose-stimulated hepatic glucokinase induction is progressively delayed in time of onset from about 4 to 11 hours as Sprague-Dawley rats age from 2- to 24-months (19). Since physiological doses of glucagon inhibit glucose-stimulated adaptations of hepatic glucokinase (20, Table 1.), and the duration of elevated glucagon levels in the older rats is comparable to the additional time required for glucokinase adaptation to begin (Figure 1.), the progressive delay in this enzyme adaptation during aging may be an expression of differences in the availability of glucagon following glucose administration. Changes in the availability of appropriate hormonal effectors to target tissues may be a general manifestation of the aging process and lead to the progressively decreasing adaptability and survival capacity of the aging organism.

REFERENCES

1. Klimas, J. E. (1968) J Gerontol 23, 31-34.
2. Gold, G., Karoly, K., and Adelman, R.C. (1976) Biochem Biophys Res Commun 73, 1003-1010.
3. Brancho-Romero, E., and Reaven, G.M. (1977) J Am Geriatr Soc 25, 299-302.
4. Freeman, C., Karoly, K., and Adelman, R.C. (1973) Biochem Biophys Res Commun 54, 1573-1580.
5. Olefsky, J.M., and Reaven, G.M. (1975) Endocrinology 96, 1486-1498.
6. Olefsky, J.M., Johnson,J., Lin, F., Jen, P., and Reaven, G.M. (1975) Metabolism 24, 517-527.
7. Sokal, J.E. (1966) Am J Med 41, 331-341.

8. Samols, E., Tyler, J.M., and Marks, V. (1972) in "Glucagon: Molecular Physiology, Clinical and Therapeutic Implications", ed. by P.J. Lefebvre and R.H. Unger, Pergamon Press, New York, pp. 151-180.
9. Park, C.R. and Exton, J.H. (1972) Ibid., pp. 77-108.
10. Cohen, B.J., Anver, M.R., Ringler, D.H., and Adelman, R.C. (1978) Fed Proc 37, 2848-2850.
11. Aguilar-Parada, E., Eisentraut, A.M., Unger, R.H. (1969) Am J Med Sci 257, 415-419.
12. Sharma, C., Manjeshwar, R., and Weinhouse, S.J. (1963) J Biol Chem 238, 3840-3845.
13. Müller, W.A., Faloona, G.R, Aguilar-Parada, E., and Unger, R.H. (1970) N Engl J Med 283, 109-115.
14. Grey, N., McGuigan, J.E., and Kipnis, D.M. (1970) Endocrinology 86, 1383-1388.
15. Holst, J.J., Galbo, H., and Richter, E.A. (1978) J Clin Invest 62, 182-190.
16. Park, C.R., Lewis, M.D., and Exton, J.H. (1972) Diabetes 21, 439-446.
17. Lockwood, D.H., and East, L.E. (1978) Diabetes 27, 589-591.
18. Kalish, M.I., Katz, M.S., Pineyro, M.A., and Gregerman, R.I. (1977) Biochim Biophys Acta 483, 452-466.
19. Niemeyer, H., Perez, N., and Rabajille, E. (1966) J Biol Chem 241, 4055-4059.