Vol. 87, No. 4, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 27, 1979 Pages 1207-1213

ALTERED REGULATION OF INSULIN SECRETION IN ISOLATED ISLETS OF DIFFERENT SIZES IN AGING RATS[1]

Akio Kitahara and Richard C. Adelman[2]

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

Received March 15,1979

Summary: Glucose-stimulated secretion of immunoreactive insulin (IRI) was examined in perifused pancreatic islets of Langerhans of different sizes isolated from fed and fasted, Sprague-Dawley rats aged 2- to 24-months. Small and large islets show distinctly different biphasic secretory responses which are modified during starvation or aging. The rate of IRI secretion is several fold greater in large than in small islets, irrespective of donor age or nutritional status. Starvation severely reduces the rate of IRI secretion, essentially abolishing the stimulatory effect of glucose in small islets from fasted, old rats. Aging inhibits IRI secretion, but to a far greater degree in small than in large islets. The increased number of large islets in the pancreas of aging rats may represent a physiological compensatory response to the diminished functional capability of small islets with respect to glucose-stimulated secretion of IRI.

Introduction

Effects of aging on the regulation of IRI levels *in vivo* and on the secretion of IRI by isolated, pancreatic islets of Langerhans were reported previously(1). Unfortunately, however, this investigation utilized islets of undesignated size. It now is evident that the size-distribution of islets of Langerhans changes during aging in rats(2-6). The present article indicates that the nature of glucose-stimulated IRI secretion *in vitro* depends on islet size, as well as age and nutritional status of donor rats.

Methods

Animals- Specific pathogen-free, barrier-maintained, male Sprague-Dawley rats aged 2- to 24-months were obtained from a special colony maintained for R.C. Adelman at the Charles River Breeding Laboratories. Rearing conditions there and preliminary evaluation of pathology(7), as well as survival data

[1] Supported in part by Research Grants AG-00368 and CA-12227 from the NIH, and an Established Investigatorship of the American Heart Assoc. to R.C.A.

[2] To whom inquiries should be addressed at the Temple University Institute on Aging, 3420 N. Broad Street, Philadelphia, Pennsylvania 19140.

0006-291X/79/081207-07$01.00/0

Copyright © 1979 by Academic Press, Inc.
All rights of reproduction in any form reserved.

and conditions for short-term maintenance prior to experimentation in our animal facility(8), were described previously. Rats either were fasted for 72 hours or fed ad libitum a pasteurized Charles River Chow which is of constant percent composition and component source, with free access to drinking water. Male, HLA Hartley guinea pigs weighing 500-600g were obtained from Hilltop Lab animals.

Reagents- Rat insulin was purchased from the NOVO Research Institute (Copenhagen); porcine Iletin insulin from Eli Lilly; [^{3}H]leuine (specific activity 40-60 Ci/m mole) and porcine [^{125}I]monoiodoinsulin (specific activity 102 μCi/μg) from New England Nuclear; Sephadex G-50 and Sepharose 4B from Pharmacia Fine Chemicals; bovine serum albumin fraction V from Miles Laboratories; and collagenase IV from Worthington.

Islet Preparation and Perifusion- Islets were isolated from fed or fasted rats aged 2- to 24-months, and were perifused with buffered solutions of glucose according to the procedure of Lacy and coworkers(9). Islets were designated small or large, corresponding to approximate diameters of 50-80 and 350-400 μm, respectively, as measured under a dissecting microscope. Fifty to 100 small or large islets were perifused at a constant flow rate of 0.7-0.8 ml/min. The concentration of glucose in the perifusion medium initially was maintained at 1.67 mM for a 1-hour equilibration period prior to designation of zero time. Subsequently, the glucose concentration was increased to 16.7 mM and maintained at that level for two hours. At the indicated flow rate, all changes of glucose concentration in the perifusion medium were achieved completely within 6 minutes.

For the isotope incorporation studies, 100 islets at a time were perifused as described above except that 2μCi/ml of [^{3}H]leucine were added to the 16.7 mM glucose at zero time. Perifusates were collected at 30-minute intervals, incubated with excess anti-insulin serum bound to cyanogen bromide-activated Sepharose (preparation described below), treated as described by Kaelin et al.(10), and assayed for radioactivity in a liquid scintillation counter.

IRI Assay- Specific antisera against purified porcine insulin(11) was developed in guinea pigs by the method of Vaitukaitis et al.(12). Anti-insulin Sepharose was prepared with a selective antiserum as described by Berne(13), and was utilized in a solid-phase radioimmunoassay according to the method of Wide(14), using purified rat insulin as a standard. Radioactivity of [^{125}I] insulin was determined in an automatic gamma counter.

Results

Small Islets- The time course and magnitude of the IRI secretory response by isolated pancreatic islets of Langerhans to an elevation of the glucose concentration in the perifusion medium depend on the size of the islet and the nutritional status and age of the donor rats. As illustrated in Figure 1 for small islets from 2-month old fed rats, there are three distinct features of the secretory response over a period of two hours: 1) a small initial rise in the rate of IRI secretion at 3-7 minutes; 2) a second, more prominent

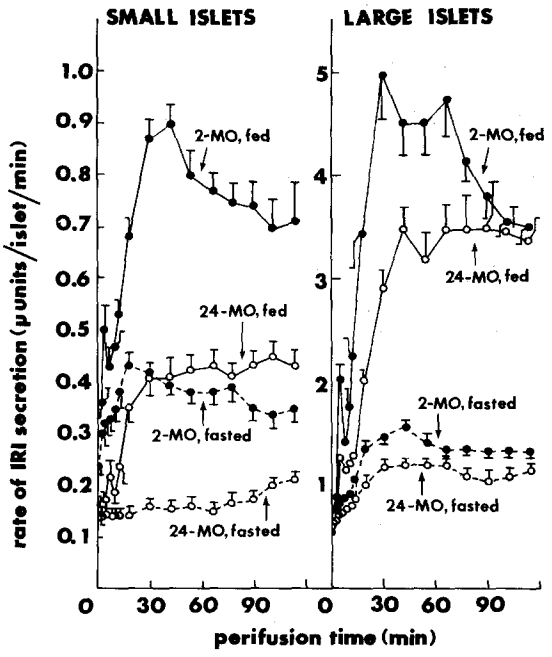

FIG.1. EFFECTS OF AGING, NUTRITIONAL STATUS AND ISLET SIZE ON GLUCOSE-STIMULATED SECRETION OF IRI.

The rate of IRI secretion is calculated from the concentration of IRI in serially collected perifusates of islets of the indicated sizes isolated from rats of the indicated ages and nutritional status. Each value represents the mean ± standard error for 4 islet preparations. Each preparation consists of 50-100 islets collected and pooled from separate groups of 2-3 rats.

burst that begins at 9-12 minutes and peaks at about 30-40 minutes; and 3) a plateau or gradually declining rate of secretion at about 40-120 minutes.

With small islets from 24-month old fed rats, the unstimulated rate of IRI secretion is approximately 50% of that observed with the islets from 2-month old rats. The initial and second bursts of IRI secretion in response to elevation of the glucose concentration occur at approximately the same times and extent of stimulation by islets from rats of both ages, although the actual stimulated rates of IRI secretion are about 2-fold greater by islets from the younger rats.

Starvation for 72 hours reduces the stimulated rate of IRI secretion. Also, starvation apparently abolishes the initial phase of secretion. The

second phase of the response in islets from young fasted rats occurs at approximately the same time as in islets from young fed rats. In small islets from 24-month old fasted rats, the stimulated rate of IRI secretion is negligible during the first 1-1.5 hours, but increases slightly at 1.5-2 hours.

Large Islets- As illustrated in Figure 1 for large islets from 2-month old fed rats, the three distinct features of glucose-induced IRI release are similar to those of small islets from 2-month old fed rats. However, the stimulated rate of IRI secretion is approximately five times greater from larger than from small islets. Aging is accompanied by a small decrease in the stimulated rate of IRI secretion, but only during the first 60 minutes of response.

In large islets from fasted rats of both ages, two effects of starvation are apparent: 1) an overall reduction in the stimulated rate of IRI secretion relative to large islets from fed rats; and 2) a virtually abolished initial phase of stimulated IRI secretion. Aging is accompanied by at most only a slight reduction in the stimulated rate of IRI secretion.

Total Secretion of IRI- The total amounts of IRI secreted in response to glucose for a 2-hour period in vitro by small or large islets from young or old rats that were fed or fasted are indicated in Table 1. The total amounts of IRI secreted from large islets exceeds that from small islets by a factor of four to eight. Neither the age nor the nutritional history of the donor rats affects this general trend. The total amounts of IRI secreted from islets of fed rats exceeds by two to three times that from islets of fasted rats. This difference is independent of the effects of islet size or donor age. Aging is accompanied by a reduction in the total amounts of IRI secreted, although the phenomenon is more pronounced in small islets from fed or fasted rats than in large islets.

Secretion of Newly Synthesized IRI- The incorporation in vitro of [^{3}H]leucine into IRI secreted by small islets from 2- to 24-month old fasted

Table 1. TOTAL SECRETION OF IRI

Age (months)	Nutritional Status	Total IRI Secreted (μunits/islet/2 hours) Small Islets	Large Islets
2	fed	88.5±3.7	464±30
2	fasted	45.6±2.8	161±2.5
24	fed	46.1±6.7	361±29
24	fasted	19.4±1.0	131±9.7

Total IRI was calculated by adding the amounts secreted serially into collected fractions of the perifusate during a 2-hour period, as described in the legend to Figure 1. Each value represents the mean ± standard error for 3-4 islet preparations. Each preparation consists of 50-100 islets collected and pooled from separate groups of 2-3 rats of the indicated age and nutritional status.

rats is indicated in Table 2. The time course of appearance of radioactive secretory products which bind to insulin antibodies is identical by islets from each age group, detectable initially at approximately 60 minutes following elevation of the glucose concentration in the perifusion medium. The amount of radioactive IRI secreted by islets from 2-month old rats slightly exceeds that from 24-month old rats. Although data are not presented, no immunoreactive radioactivity is detectable during the 2-hour period if the glucose concentration in the perifusion medium is maintained at 1.67mM.

Discussion

The secretion of IRI by isolated pancreatic islets of Langerhans perifused with glucose has been extremely well characterized by numerous investigators in terms of kinetics of release, role of hormone synthesis and metabolism, effects of nutritional status, etc.(e.g., 15). Patterns of glucose-stimulated secretion of IRI by small islets described above for a 2-hour period, the experimental conditions most frequently exploited previously, are in good agreement with earlier studies. For example, abolishment of the early phase of stimulated IRI secretion by islets from fasted young rats is virtually idential to the findings of Rabinovitch et al.(16).

Increasing age of donor rats complicates matters enormously, since this is accompanied by a shifting distribution of islet populations which are

Table 2. INCORPORATION OF [^{3}H]LEUCINE INTO SECRETED IRI

Time	Radioactivity (dpm/islet)	
	2-month	24-month
0	0	0
30	0	0
60	0.36±0.05	0.24±0.03
90	1.54±0.03	1.26±0.05
120	1.78±0.04	1.54±0.03

Radioactivity was determined for insulin antibody-bound material secreted by small islets from fasted rats of the indicated ages following perifusion with 16.7 mM glucose, as described in the legend to Figure 1. Each value represents the mean ± standard error for 3 islet preparations. Each preparation consists of 100 islets pooled from separate groups of 2-3 rats.

heterogeneous with respect to size (2-6). Apparently, each of these islet populations may be characterized by quantitative and/or qualitative differences in susceptibility to regulation of IRI secretion. With respect to aging, the most dramatic difference concerns the inability to stimulate secretion of IRI by small islets from fasted, 24-month old rats. Although the mechanism is not yet understood, it probably relates to an inability to mobilize molecules of IRI that were synthesized prior to isolation of the islets. Molecules of IRI which are newly synthesized in vitro are secreted by small islets from young and old fasted rats at the same time, after approximately one hour of perifusion with the elevated concentration of glucose. It is not possible to evaluate the rate or magnitude of secretion of newly synthesized IRI in the absence of data on pool size and specific radioactivity of leucine within the beta cells of the islets.

The initial rise in the concentration of IRI in portal vein blood of fasted rats treated with glucose is not impaired as rat age increases from 2- to 24-months(1). The ability of isolated small pancreatic islets to increase the rate of IRI secretion in vitro during the first two hours of stimulation by glucose perifusion is virtually abolished during this portion of the lifespan of donor rats. In contrast, inhibitory effects of aging on glucose-stimulated secretion of IRI in vitro by large islets are only very

slight. Therefore, it is tempting to speculate that the increased number of large islets in the pancreas of aging rats represents a compensatory response to the diminishing responsiveness of small islets. In this manner, the large islets may assume the major, if not total, responsibility for short-term adaptive secretion of IRI in response to fluctuating levels of blood glucose.

References

1. Gold, G., Karoly, K., Freeman, C., and Adelman, R.C. (1976) Biochem. Biophys. Res. Commun. 73, 1003-1010.
2. Hellman, B. (1959) Acta Endocrinol. 31, 91-106.
3. Hajdu, A., and Rona, G. (1967) Diabetes 16, 108-110.
4. Remacle, C., Hauser, N., Jeanjean, M., and Gommers, A. (1977) Exper. Geront. 12, 207-214.
5. Gold, G., Reaven, E., and Reaven, G. (1978) Geront. Soc. Proc., November, p. 77.
6. Kitahara, A., Obenrader, M., Rosenfeld, A., Burch, T., and Adelman, R.C. (1978) Geront. Soc. Proc., November, p. 88.
7. Cohen, B.J., Anver, M.R., Ringler, D.H., and Adelman, R.C. (1978) Fed. Proc. 37, 2848-2850.
8. Britton, G.W., Rotenberg, S., Freeman, C., Britton, V.J., Karoly, K., Ceci, L., Klug, T.L., Lacko, A.G., and Adelman, R.C. (1975) Explorations in Aging, V.J. Cristofalo, J. Roberts and R.C. Adelman, eds., pp. 209-228, Plenum Press, New York.
9. Lacy, P.E., Walker, M.M., and Fink, C.J. (1972) Diabetes 21, 987-998.
10. Kaelin, D., Renold, A.E., and Sharp, G.W.G. (1978) Diabetologia 14, 329-335.
11. Davoren, P.R. (1962) Biochim. Biophys. Acta 63, 150-153.
12. Vaitukaitis, J., Robbins, J.B., Nieschlag, E., and Ross, G.T.A. (1971) J. Clin. Endocrinol. 33, 988-998.
13. Berne, C. (1975) Endocrinol. 97, 1241-1247.
14. Wide, L. (1973) Radioimmunoassay Methods, K.E. Kirkham and W.M. Hunter, eds., pp. 405-412, Churchill and Livingstone, Edinburgh and London.
15. Malaisse, W.J. (1972) Handbook of Physiology, Section 7, Volume 1, D.F. Steiner and N. Freinkel, eds., pp. 237-260, American Physiological Society, Washington, D.C.
16. Rabinovitch, A., Grill, V., Renold, A.E., and Cerasi, E. (1976) J. Clin. Invest. 58, 1209-1216.