Age-Related Changes in Pancreatic Islet Cell Gene Expression

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Previous studies have indicated that insulin secretion in response to glucose diminishes with age but insulin synthesis and gene transcription do not. To determine whether expression of genes other than those that encode insulin are subject to age-related changes that could alter pancreatic islet function, mRNAs for insulins I and II, amylin, glucose transporter 2 (GluT2), glucagon, and glucokinase were quantified in 2-, 6-, 12-, and 24-month-old Fischer 344 rats using species-specific ribonuclease (RNase) protection assays. There was only a modest (1.2- to 1.3-fold) increase in insulin I and insulin II mRNAs between ages 2 and 12 months. There were no statistically significant changes in levels of glucokinase mRNA with age. In contrast, the abundances of amylin, GluT2, and glucagon mRNAs all doubled during the same period. Variance in values from 24-month-old rats was too great to allow conclusions, except that the ratio of insulin II mRNA to insulin I mRNA increased with age. This change was not related to islet mass or total insulin mRNA abundance because it persisted at age 24 months, when total mRNA abundance had decreased. These results indicate that aging is associated with significant alterations in the relative proportion of expression of pancreatic islet cell genes implicated in insulin secretion and in intraislet glucose metabolism.

IT IS GENERALLY ACCEPTED that aging is commonly associated with impaired glucose tolerance.[1,2] Although resistance to insulin action accounts for most of the observed impairment in glucose tolerance,[3] there are also apparent age-related changes in insulin secretion[3-5] and clearance.[2-4,6-8] Any inconsistencies in findings concerning insulin secretion between studies are most likely explained by difficulties in distinguishing the effects of age per se from those of diseases and pathologies that are commonly found in aged groups.[9]

In recent years, genes that encode several components unique to pancreatic β cells important for modulation of insulin synthesis and secretion[10-13] have been cloned and sequenced. The effect of age on expression of these genes remains unknown. To determine whether aging alters the coordinate expression of these several genes involved in insulin secretion and glucose homeostasis, we analyzed pancreas taken from male Fischer 344 rats at different ages for the steady-state content of insulin I and II, amylin, glucose transporter (GluT2), glucagon, and glucokinase mRNAs. We found that abundance of amylin, GluT2, and glucagon mRNAs all doubled between 6 and 12 months of age, whereas insulin mRNA abundance changed only minimally, and glucokinase mRNA, not at all.

Of particular interest for studies on aging, proportions of mRNAs that encode each of the two nonallelic insulins present in rats insulins I and II changed with age. Insulin I comprised 55% of total insulin mRNA in 2-month-old rats and decreased to 48%, 47%, and 42% of total insulin mRNA in 6-, 12-, and 24-month-old rats. These changes were not related to total insulin mRNA abundance, rat weight, or any parameter measured other than age per se. Thus, altered ratios of the two mRNAs that encode insulin in rats may serve as a useful marker for aging at the molecular level.

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MATERIALS AND METHODS

Animals

Male Fischer 344 rats aged 2, 6, 12, and 24 months were obtained from the National Institute of Aging Colony maintained by Harlan Industries, Indianapolis, IN. All rats had a stabilized food intake within 3 days of arrival, and experiments were performed after 1 week's maintenance of the rats at our facility under standard laboratory conditions with ad libitum feeding of laboratory rat chow (Teklad, Madison, WI) and tap water. Rats were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally) and were killed between 1 and 3 PM by exsanguination after excision of the pancreatic tissue. Internal organs of each rat were inspected for gross pathology. Animals bearing tumors were discarded from the analysis. Serum creatinine level was determined to exclude rats with renal failure.

RNA Preparation and Analysis

Frozen pancreases were pulverized on dry ice, lyophilized, and solubilized in guanidine thiocyanate, and RNA was prepared by centrifugation through $CsCl_2$. RNA concentration and integrity were analyzed for each sample as described previously.[14-17] Briefly, to control for potential sample-to-sample variation in RNA concentration and to ensure RNA integrity, aliquots (5 mg) of each RNA sample used in ribonuclease (RNase) protection analyses were electrophoresed on denaturing formaldehyde-agarose gels, stained with ethidium bromide, and photographed with Polaroid (Cambridge, MA) positive-negative film, and the 28S bands were submitted to scanning densitometry using a BioRad model 620 Videodensitometer (Richmond, CA). Results were used to normalize data from RNase protection experiments reported later, as described previously.[16] Data were normalized to 28S RNA rather than to measurements of actin or tubulin because of reports that those mRNAs can be altered by insulin or metabolic perturbations in vivo[18,19] and by insulin in vitro.[20]

Complementary (antisense) RNA probes used to analyze mRNAs for rat insulins I and II, mRNA precursors for rat insulins I and II, and glucagon, amylin, GluT2, and glucokinase mRNAs have been described previously.[14,15,17,21-24]

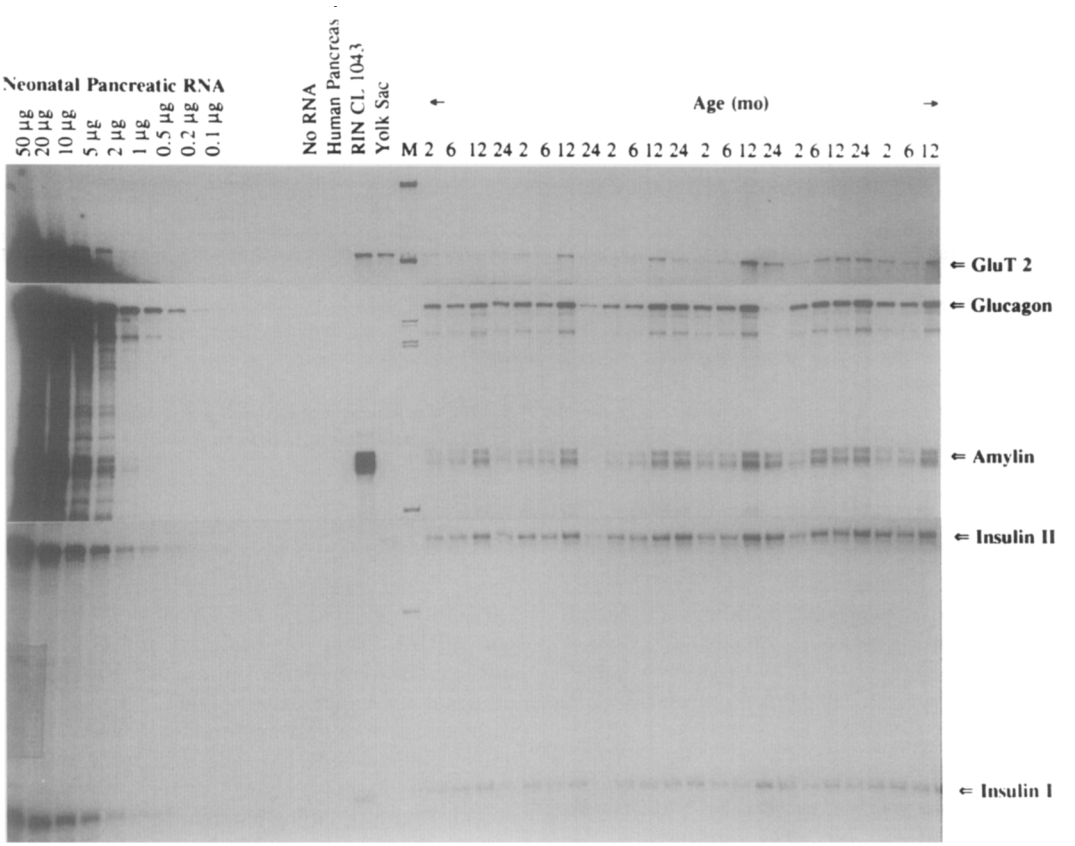

Fig 1. Measurement of GluT2, glucagon, amylin, and insulin I and II mRNAs in a single RNase protection assay. RNA was hybridized with uniformly labeled RNA probes complementary to rat insulin I and II, rat amylin, rat glucagon, and rat GluT2. Markers (lane M) are 35S-labeled *HinfI* digestion fragments of pSP65; sizes are 512, 396 (doublet), 354 (doublet), 218, and 176 bases. Yolk Sac lane shows products from hybridization to rat yolk sac RNA, and RIN CL 1043 lane, to RNA from a rat insulin-producing cell line (RIN 1046-43). Lanes at far left show results from hybridizations to serial dilutions of rat neonatal day–14 pancreatic RNA. Amounts of RNA are shown above individual sample lanes. Autoradiogram is a composite of different exposures of the same gel. Exposure varied from 1 week for region containing bands protected by GluT2 mRNA to 2 hours for portion showing bands protected by insulins I and II.

RNase Protection Assays

RNase protection assays were performed exactly as described for insulin mRNA and insulin mRNA precursors.[14,17] Amounts of RNA used for each analysis are indicated in Figs 1 and 2. Each assay was validated using methods illustrated for glucokinase mRNA, which is shown in Fig 2. Serial (2- to 2.5-fold) dilutions of synthetic mRNAs (data not shown) and serial (2- to 2.5-fold) dilutions of control rat pancreatic RNA were assayed in parallel with experimental samples. Human pancreatic RNA was included as a negative control in each assay. Repeated autoradiographic exposures were performed until densities of experimental bands were within the linear range provided by the controls. Correlation coefficients for standard curves were ≥.99 for each assay. Assay variation is typically ±10% on repeat analysis. Each probe gave a protected fragment with size predicted by probe construction. Each probe contained a plasmid-derived sequence so that residual undigested probe, if present in experimental samples, could be distinguished by size from probe protected from digestion by hybridization to mRNA. No probe gave protected fragments of appropriate size when hybridized to human RNA, which demonstrates species-specificity. Glucokinase probe was constructed so that mRNA isoforms produced by alternate splicing could not be

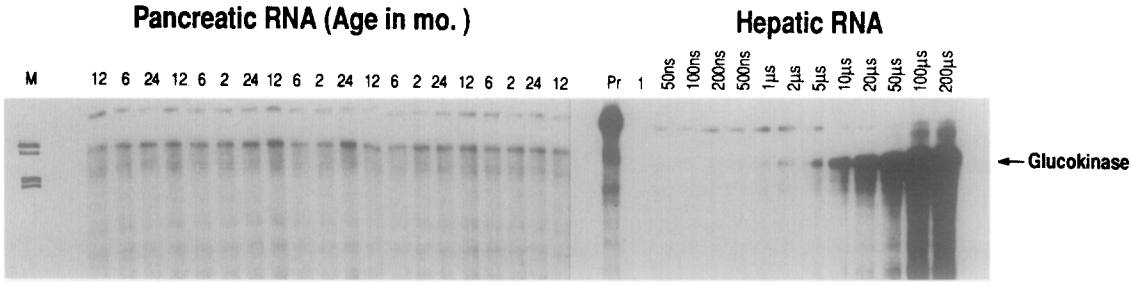

Fig 2. Measurement of glucokinase mRNA. Aliquots (700 µg) of total pancreatic RNA were hybridized to uniformly labeled RNA probe complementary to 400 bases of rat glucokinase. Serial dilutions of hepatic RNA, used as controls, are shown on the right.

Table 1. Quantitation of mRNAs That Encode Insulins I and II, Amylin, Glucagon, GluT2, and Glucokinase

Age (mo)	mRNA					
	Insulin I	Insulin II	Amylin	GluT2	Glucagon	Glucokinase
2	100 ± 8	100 ± 12	100 ± 11	100 ± 16	100 ± 12	100 ± 12
6	103 ± 6*	109 ± 5†	134 ± 15‡	95 ± 13	96 ± 17	84 ± 8
12	123 ± 5§‖	136 ± 5§¶	194 ± 13§#**	182 ± 31**††	183 ± 13§#‡‡	92 ± 16
24	99 ± 16	129 ± 15	147 ± 32	175 ± 43	134 ± 43	130 ± 23

NOTE. Multiple, differently timed exposures of the gel shown in Fig 1 and a 1-month exposure of the gel shown in Fig 2 were submitted to scanning densitometry. Results are plotted as the percent change from the mean of values for each mRNA at age 2 months.

*> glucokinase at 6 months, $P < .05$; < amylin at 6 months, $P < .05$.

†> glucokinase at 6 months, $P < .01$.

‡> GluT2 at 6 months, $P < .05$; > glucokinase at 6 months, $P < .05$.

§> the same mRNA at 2 and 6 months, $P < .01$.

‖< amylin and GluT2 at 12 months, $P < .005$; < glucagon at 12 months, $P < .025$; > glucokinase at 12 months, $P < .05$.

¶< amylin and GluT2 at 12 months, $P < .005$; < glucagon at 12 months, $P < .05$; > glucokinase at 12 months, $P < .01$.

#> insulin I or II mRNA at 12 months, $P < .05$.

**> glucokinase at 12 months, $P < .005$.

††> GluT2 mRNA at 6 months, $P < .01$.

‡‡> glucokinase at 12 months, $P < .01$.

distinguished. These include the presence or absence of a noncoding cassette exon (2a) and the presence or absence of 51 bases at the beginning of exon 4.[23] Because these represent minor species in pancreas,[23] their inclusion should not affect the interpretation of results.

Statistical Analyses

All experimental data are presented as the means ± SE of five to six rats. Statistical analyses were performed using unpaired Student's *t* tests.

RESULTS

Measurement of multiple pancreatic islet mRNA levels in a single RNase protection assay is shown in Fig 1. RNA probes were complementary to rat insulin I and II, rat amylin, rat glucagon, and rat GluT2. Measurement of glucokinase mRNA level is shown in Fig 2. Bands from assays illustrated in Figs 1 and 2 were quantified as described earlier. Results are listed as the percent change from the mean value for each mRNA at age 2 months in Table 1. There were no statistically significant changes in glucokinase mRNA levels with age. Values for all other mRNAs were greater in rats at 12 months as compared with 2 or 6 months. Abundance of amylin mRNA was significantly greater at 6 than at 2 months, and also was increased significantly between 6 and 12 months. Glucagon and GluT2 mRNAs remained constant between 2 and 6 months, but doubled between 6 and 12 months. There was a modest but statistically significant increase in insulins I (23%) and II (36%) between ages 2 and 12 months. Increases in amylin, GluT2, and glucagon mRNAs between 2 and 12 months were all significantly greater than increases in insulin I and II mRNAs. At age 12 months, increases were greater than for insulin I, insulin II, and glucokinase mRNAs. The increments between 2 and 12 months in amylin, glucagon, and GluT2 mRNAs were not significantly different from one another.

As evident from inspection of Figs 1 and 2, there was great interindividual variability in abundances of these several islet-specific mRNAs in 24-month-old animals. This variability appeared greater for amylin, GluT2, and glucagon mRNAs than for insulins I and II and glucokinase mRNAs. In some samples, there was almost complete loss of signal for either amylin, GluT2, or glucagon mRNAs, with persistent or only moderately reduced levels of insulin and glucokinase mRNAs. These differences did not appear related to plasma glucose, weight, or apparent state of overall health of individual animals. These variations cannot be due to assay artifact, because all mRNAs were analyzed using the same pancreatic RNA sample.

As illustrated in Fig 3, there was a significant change in the proportion of the two insulin mRNAs with age ($r = .65$, $df = 23$, $P < .001$). This change was not correlated with insulin mRNA abundance and persisted and increased in 24-month-old animals, despite the greater interanimal variability in total insulin (and other) mRNA abundance in this group. This result could be caused by age-dependent changes in specific transcription factors that have differing affinities for insulin I and II gene promoter sequences. Alternatively, they could be due to epigenetic modifications

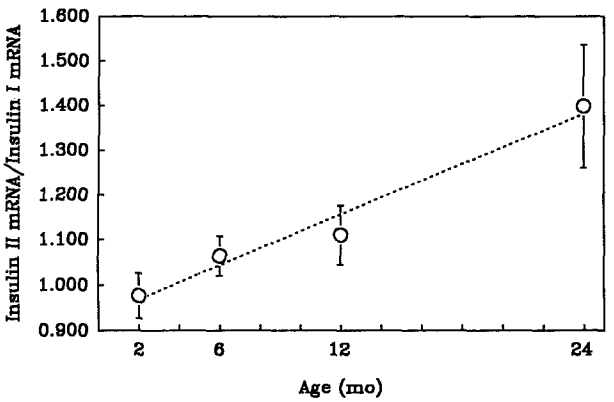

Fig 3. Change in proportions of insulins I and II mRNAs with age. The ratio of insulin I to insulin II mRNA was calculated from data listed in Table 1 and is plotted as a function of age. The ratio of insulin II to insulin I expressed as a function of age had a correlation coefficient of $r = .65$ on 23 *df* and was significant at $P < .001$.

of DNA that accumulate with age or factors that control mRNA half-life. This change would appear to be a reasonable candidate for further evaluation as a molecular marker for aging, given the overall similarity in structure and expression of the two genes.

DISCUSSION

Previous studies have shown that the amount of insulin secreted in response to a given increment in plasma glucose per islet β cell decreases with age in rats.[4,7] This decrease may be compensated for by β-cell hypertrophy, hyperplasia, or an increase in numbers of islets. Because the secretory response to other insulin secretagogues including glyceraldehyde[25] is maintained, the decrease in secretory response is likely associated with a decrease in the ability of the β cell to take up and/or metabolize glucose to trioses. These changes could be caused by changes in transcription levels of genes encoding β-cell glucose transporter and/or β-cell glucokinase. The finding that GluT2 mRNA actually increases with age rules out the former possibility. Although decreases in GluT2 mRNA are associated with the secretory defect in non–insulin-dependent diabetes both in humans and in animal models,[26] there is no loss of GluT2 mRNA with age. The present study shows a lack of change in glucokinase mRNA despite increments in other mRNAs measured. Thus, it appears that there is a relative reduction of glucokinase mRNA with age. This may explain in part the decrease in islet responsiveness to glucose in aging animals. The relatively greater increments in basal levels of glucagon, amylin, and GluT2 mRNAs may reflect differential responses of these genes to trophic signals that cause islets to compensate for the decreased insulin-secretory response to glucose that occurs with age. Similar to previously reported findings in rats,[7] we find that insulin mRNA abundance increases only minimally with age.

The greater increment in amylin than in insulin mRNA suggests that the proportions of insulin and amylin synthesized and secreted may change with age, as appears to occur in insulin-resistant states.[27,28] The reverse occurs in fetal and neonatal rats, where amylin mRNA is proportionately lower than in 2-month-old animals (unpublished observation, August 1993).

In summary, this study shows that aging is associated with significant alterations in the relative proportions of mRNAs encoded by several genes expressed specifically in pancreatic islet cells. These changes may represent more or less successful adaptations to age-related alterations in β-cell sensitivity to glucose. They also may increase resistance to peripheral insulin action by increasing the amount of amylin co-secreted with insulin[28] with age. Because of these several features, our findings warrant further study.

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