Effects of Hypoglycemia and Prolonged Fasting on Insulin and Glucagon Gene Expression

Studies with In Situ Hybridization

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Abstract

In situ hybridization of proinsulin and proglucagon mRNA was performed in rat pancreas to assess prohormone gene expression during various glucopenic conditions. During a 4-d fast mean blood glucose declined by 48 mg/dl; proinsulin mRNA signal density remained normal while proglucagon mRNA signal density more than doubled. At the end of a continuous 12-d insulin infusion blood glucose averaged 53±12 mg/dl; proinsulin mRNA signal density declined to 30% of controls while proglucagon mRNA signal density more than doubled. In insulinoma-bearing NEDH rats blood glucose averaged 34±3.5 mg/dl; the proinsulin mRNA signal was virtually undetectable and proglucagon mRNA signal density was more than twice the controls. There was no detectable change in either β-cell area or islet number in rats subjected to fasting or insulin infusion, but in insulinoma-bearing rats β cell area was markedly reduced. Thus compensation during 4 d of starvation involves an increase in glucagon gene expression without change in insulin gene expression or β cell mass. In moderate insulin-induced hypoglycemia glucagon gene expression is increased and insulin gene expression decreased. In more profound insulinoma-induced hypoglycemia, in addition to the foregoing changes in hormone gene expression, there is a profound reduction in the number of insulin-expressing cells.

Introduction

Delivery of adequate amounts of fuels to the brain is an essential physiologic function. Because the brain utilizes only glucose and ketones, hepatic glucose and ketone production must be enhanced and insulin-mediated glucose uptake reduced in order to meet cerebral fuel requirements during a reduction in availability of glucose. Such metabolic adjustments are in fact achieved whenever the concentration of glucagon is high and that of insulin low (1).

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It has previously been reported that glucopenia resulting from prolonged fast (2, 3), phloridzin treatment (4), and insulin administration is associated with an increase in plasma glucagon levels (4) and a decrease in endogenous insulin secretion (5). However, it is not known if such changes are the consequence of an alteration in hormone gene expression or in the quantity of cells that express these hormones or both. In this report we address this issue by means of in situ hybridization histochemistry, a technique that has recently been found useful for both quantitation of the number and area of islets and of the density of their proinsulin and proglucagon mRNA (6).

Methods

Experimental groups and designs. Pancreata were obtained from Wistar rats subjected to a 4-d fast with water ad lib. or to a 12-d insulin infusion. The body weight of the fasted rats changed from 290±10 to 220±10 g. The age-matched control rats weighed 297±11. In the fasted group blood glucose averaged 137.8±6.0 mg/dl before the fast and 89±9 at the time of sacrifice. In control rats the final blood glucose averaged 131.7±3.1 mg/dl. Pancreata were also obtained from insulinoma(RINm38)-bearing New England Deaconess Hospital (NEDH) rats (7) after at least 9 d of severe hypoglycemia. This tumor is syngeneic in the NEDH rat and is produced by x-irradiation. It produces insulin and somatostatin but not glucagon. In insulin-infused rats hypoglycemia of about 50 mg/dl was maintained by infusing regular porcine insulin (Eli Lilly Corp., Indianapolis, IN) at a rate of 1 to 2 U/24 h through a silastic tubing (Dow Corning Corp., Midland, MI) previously implanted in an external jugular vein under sodium pentothal anesthesia.

In situ hybridization. All pancreata were excised under anesthesia and processed for in situ hybridization as described by Han et al. (8). Pancreata from age-matched Wistar control rats were included in each hybridization run. Pancreata were divided into head (that portion of pancreas attached to gastrointestinal tract) and tail (that portion not in contact with gastrointestine). Sections of 15-μm thickness were prepared in a cryostat and hybridized in situ using the method of Han et al. (8) 27-mer oligonucleotide probes for proinsulin I and proglucagon (6) were end-labeled with ^{32}P (9). Specific activities of the labeled probes ranged from 2,000 to 3,000 Ci/mmol (1 Ci = 37 GBq). From each pancreas two tissue sections of pancreatic head and four of pancreatic tail were hybridized with each probe. Prohormone mRNA signal density (μm^2 of signal caused by silver grains/per μm^2) was quantitated under light microscopy by digitizing dark field images (10×) of radioautographs from four sections of each pancreas for each probe using a videometric 150 image analyzer (American Innovision Inc., San Diego, CA). Islet area was determined by tracing with the screen cursor the boundaries of every islet under bright field. The number of islets were measured using the proinsulin mRNA signal as the marker.

Table I. Density of Specific Prohormone mRNA (Signal per Islet Area–Islet Area Was Determined Independently of mRNA Signal by Direct Examination under Bright Field), Percent Islet Area (Endocrine/Exocrine) and Number of Islets per mm² of Pancreas

Group	Region	mRNA Signal/Islet Area		Morphometry	
		Proinsulin	Proglucagon	Islet area	Islets
				per mm²	
Normal	Tail	60.5±3.5	19.7±3.8	1.75±0.54	1.39±0.20
$n = 7$					(0.69±0.04)
	Head	24.2±5.5	NA	1.06±0.33	1.04±0.33
Insulin Infusion	Tail	18.3±1.0*	47.8±2.7*	1.70±0.30	1.31±0.62
$n = 3$	Head	TL	NA	TL	TL
4-d fast	Tail	57.0±3.8	46.8±0.9*	1.70±0.28	1.50±0.19
$n = 3$	Head	24.6±1.0	NA	1.64±0.10*	1.69±0.41
Insulinoma	Tail	3.6±2.4*	52.1±14.6*	0.09±0.05*	0.29±0.18*
$n = 3$				(1.06±0.04*)	(0.65±0.12)
	Head	TL	NA		

*$P < 0.01$. TL, signal was too low to permit quantitation. NA, α cells are too sparse in this region of the pancreas to permit quantitation. Measurements in parentheses are based on proglucagon mRNA signal.

Every islet in the four sections was measured. Area of the pancreatic section was measured by setting the light intensity in the image analyzer so as to detect only tissue areas and to exclude space between tissue. In insulinoma-bearing rats the proinsulin mRNA signal was too low to provide an accurate measurement of the number of islets; in this group quantitation of number and area of islets is based on the proglucagon signal since the glucagon-producing α cells are located at the islet periphery.

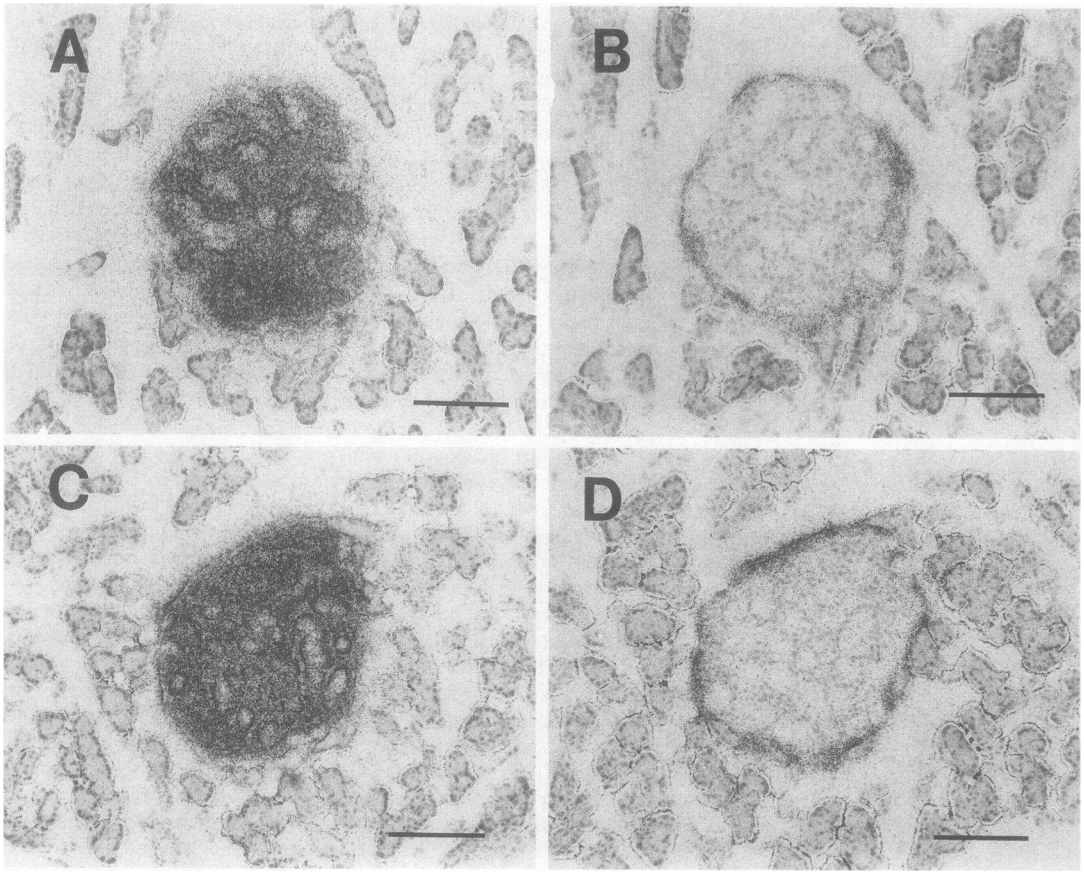

Figure 1. Bright field photomicrographs of normal (*A* and *B*) and starved (*C* and *D*) rat pancreatic sections hybridized in situ with ^{32}P-end-labeled insulin (*left*) and glucagon (*right*) oligonucleotide probes in adjacent sections. Bar, 100 μm.

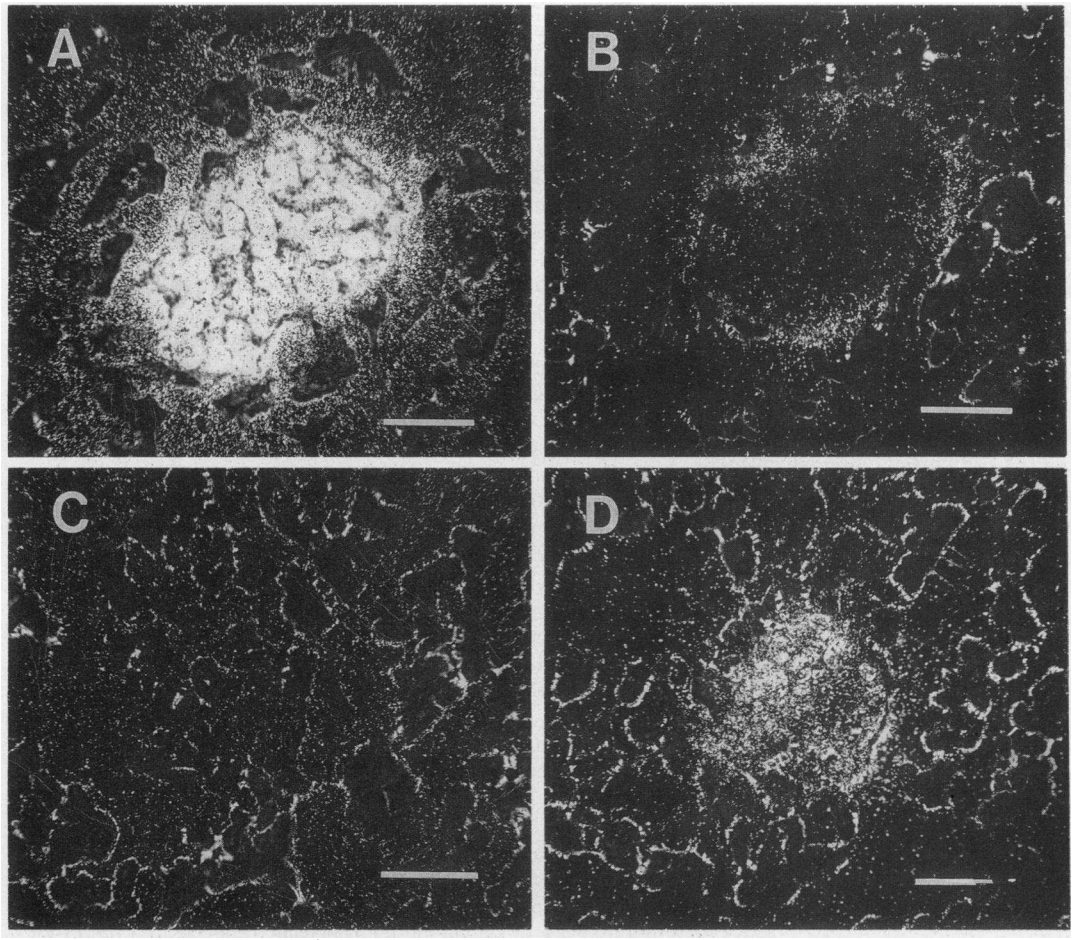

Figure 2. Dark field photomicrographs of normal (*A* and *B*) and NEDH (*C* and *D*) rat pancreatic sections hybridized in situ with ^{32}P-end-labeled insulin (*left*) and glucagon (*right*) oligonucleotide probes in the adjacent sections. Bar, 100 μm.

The results were compared with controls and significance of differences determined with the Student *t* test for two groups.

Results and Discussion

The hybridization results are shown in Table I and Fig. 1. In the pancreata of rats made mildly hypoglycemic by a 4-d fast there was no change in density of the proinsulin mRNA signal, but a 238% increase in proglucagon mRNA signal density was observed. (This was confirmed in two parallel experiments using dot-blot hybridization to quantitate total proinsulin mRNA [data not shown]). In rats maintained in moderate hypoglycemia by a 12-d insulin infusion there was a 70% reduction in proinsulin mRNA signal density (this was also confirmed by dot-blot hybridization [data not shown]). Proglucagon mRNA density averaged 243% of controls. In the severely hypoglycemic insulinoma-bearing rats there was a 94% reduction in proinsulin mRNA signal density. Proglucagon mRNA signal density was 264% of the controls. The final glucose concentrations in this group averaged 34±3.5 mg/dl.

There was no decrease in β cell area or islet number in the rats subjected to prolonged fasting or to insulin infusion, whereas in insulinoma-bearing rats the total endocrine area was reduced to 61% of the controls (Fig. 2 *D*); however, the area of proinsulin-expressing endocrine pancreas measured only 5% of the controls (0.09±0.05, $P < 0.001$), implying a significant loss of transcriptionally active β cells. The number of islets with proinsulin-expressing cells measured 21% of the controls. However, when islet number was determined using the proglucagon mRNA signal, it did not differ from the controls. Thus in the severely hypoglycemic rats the number of islets with proglucagon-expressing cells remained unchanged but the number of proinsulin-expressing cells declined dramatically.

The results indicate that during a 4-d fast hormonal compensation at the molecular level involves an increase in proglucagon gene expression without a reduction in insulin gene expression or detectable decrease in islet area or number. In fact, an unexplained increase in the area ratio was noted in the head region of the pancreas of fasted rats (Table I). Since insulin content after a 4-d fast is 40% of controls (10), a reduction in translation efficiency (11) may be a major factor in determining the rate of proinsulin biosynthesis at this time. This adjustment may permit the β cells to maintain a level of insulin secretion low enough to increase ketone production but not low enough to cause unbridled ketoacidosis (12). The reduction in insulin gene expression after 12 d of insulin infusion may reflect the effect of the moderate hypoglycemia and/or a direct inhibitory effect of hyperinsulinemia itself on insulin gene expression. Again no reduction in number or size of the pancreatic islets was noted. By contrast, the more severe chronic hypoglycemia caused by insulinoma resulted in striking β cell atrophy in addition to changes in prohormone expression. Proinsulin mRNA signal density was almost undetectable, confirming a previous study using Northern blot analysis (13). However, occasional cells containing proinsulin

mRNA were present. The density of the proglucagon mRNA signal was increased more than twofold. Since insulin suppresses glucagon gene expression (6), low concentrations of insulin within the islet microcirculation perfusing α cells may have contributed to the greatly increased proglucagon mRNA levels, as is the case in streptozotocin-induced insulin-dependent diabetes (6). The number of islets appeared to be reduced when the counts were based upon the profoundly attenuated proinsulin mRNA signal, but there was no reduction in the number of islets containing the proglucagon mRNA signal. The technique used here does not permit quantitation of area of cells expressing the proglucagon gene.

The results indicate that changes in prohormone gene expression are a part of the α and β cell responses to glucopenia and prolonged fasting, as they are in the antithetic circumstances of glucose overloading (6) and partial pancreatectomy (6). They also suggest that when changes in insulin and glucagon production fail to compensate for a dearth of cerebral fuels, a further response consisting of β cell atrophy takes place, thereby reducing any constitutive insulin release that cannot be suppressed by glucopenia.

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