Substrate-Induced Regulation of Gene Expression in the Pancreas

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(Received June 28, 1996; returned for revision January 9, 1997; accepted May 31, 1997)

Synthesis and secretion rates of pancreatic proteins, particularly of the (pro)enzymes, have been studied under different conditions and in several animal systems. This brief overview will focus on the levels of mRNA, the rates of protein synthesis and protein secretion in the pancreas under controlled dietary regimens.

INTRODUCTION

Based on the hypothesis of Pavlov [1] and early experiments with rats [2, 3], it was thought that diets rich in either fats, proteins or carbohydrates would cause a selective increase of synthesis of those pancreatic enzymes corresponding to the predominant substrates. Although correct when diets contained at least 15 percent protein, this concept had to be revised, because in extreme situations, such as a zero protein/high carbohydrate diet, the relevant enzymes (i.e., serine proteases and amylase) appeared to be inversely correlated to the amount of substrate fed [4].

The accumulated output of (pro)enzymes is the result of several distinct phases: first mRNA levels depend on the rate of transcription and the rate of mRNA degradation. Second, protein levels depend on the amount of available mRNA, the rate of translation, the potential size of the storage pool (zymogen granule size and density) and the rate of secretion. Individual phases have been analyzed with various tools, depending on availability of adequate technology. The focus of attention has been to correlate synthetic rates and actual tissue content of (pro)enzymes. Histochemical studies have allowed estimation of zymogen granule density. However, the molecular mechanisms by which the expression of pancreatic genes is regulated have so far not been elucidated in detail.

ACCUMULATION OF mRNA CODING FOR SECRETORY ENZYMES

To determine whether mRNA levels were regulated by diets, in vitro translation of mRNA was performed. Amylase and serine proteases were analyzed during a 9-day regime of isocaloric diets, which varied in their protein content [5]. With the exception of the zero protein diet, the mRNA-levels correlated well with the substrates fed, i.e., on a high-carbohydrate diet amylase-mRNA levels were high, while serine protease-mRNA was relatively low. With decreasing carbohydrate content amylase-mRNA decreased while serine protease-mRNA followed the increasing amount of protein in the diet [5]. With the zero protein diet, however, serine protease-mRNAs increased quite dramatically. In another study, both in vitro translation and dot blot analysis with cDNA probes were used to assess pancreatic mRNA-levels after high- and low-protein diets [6]. Hybridization analysis with trypsinogen, chymotrypsinogen and procarboxypeptidase corroborated the findings by

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Abbreviations: PSTI, pancreatic secretory trypsin inhibitor; FPLC, fast protein liquid chromatography; CCK cholecystokinin.
Wicker et al. [5], which concluded that the serine protease-mRNAs were upregulated after increases in dietary protein, in contrast to procarboxypeptidaseA-mRNA, which remained virtually unaffected under these conditions. Finally, mRNA-levels of anionic and cationic trypsinogen-mRNAs were compared to those of the mRNA-levels coding for the pancreatic secretory trypsin inhibitor [7]. Again, both high-protein and protein-free diets induced an increase in accumulation of trypsinogen and pancreatic secretory trypsin inhibitor (PSTI)-mRNA. In experiments using the transcription inhibitor actinomycin D, the half-life of mRNA species appeared increased for all tested trypsinogen-forms, while PSTI-II-mRNA displayed a decrease in its apparent half-life after application of a protein-free diet [8]. Thus, turnover rates do not completely account for the accumulation of mRNA. Yet, a general increase in mRNA coding for secretory proteins cannot be postulated since in a

Table 1. Adaptation of pancreatic mRNA levels in rats fed a protein-rich or a protein-deficient diet. mRNA levels were estimated either by in vitro-translation of mRNA and subsequent electrophoretic analysis or by dot-blot / Northern-blot analysis using cDNAs or oligonucleotides as probes. The length of adaptation to a given diet varied between the different authors: 9 days [5], two weeks [6], 5 days [9, 23], 14 days [24], four weeks [10], 10 days [7]. Arrows represent the regulation of mRNA levels in experimental diets relative to a protein sufficient control diet: upregulation (↑), downregulation (↓), slight but not significant up (↑) or down (↓) regulation and no significant change (→) in specific mRNA levels. The following methods were employed: a) Northern/dot-blotting with the use of a cDNA coding for the gene in question, b) an oligonucleotide designed to hybridize specifically to a portion of the mRNA or c) in vitro translation of mRNA and subsequent analysis of the proteins synthesized.

| Gene               | Method                      | Protein deficient | Protein rich | Reference |
|--------------------|-----------------------------|-------------------|--------------|-----------|
| Trypsinogen        | cDNA hybridization          | ↑                 | ↑            | [24]      |
| anionic (A1)       |                             |                   | ↑            | [7]       |
|                    |                             |                   | ↑            | [6]       |
|                    |                             |                   | ↑            | [10]      |
| Trypsinogen        | Oligonucleotide hybridization| ↑                 | ↑            | [9]       |
| anionic (A1, A2)   |                             |                   | ↑            | [9]       |
| Trypsinogen        | Oligonucleotide hybridization| ↑                 | ↑            | [9]       |
| cationic           | cDNA hybridization          | ↑                 | ↑            | [6]       |
|                    |                             |                   | ↑            | [10, 23]  |
| Chymotrypsinogen   | cDNA hybridization          | ↑                 | ↑            | [6]       |
| Elastase anionic   | cDNA                        | ↓                 | ↑            | [6]       |
|                    | Oligonucleotide hybridization| ↓                 | ↑            | [9]       |
| Elastase cationic  | Oligonucleotide hybridization| ↓                 | ↑            | [9]       |
| Serine proteases   | In vitro-translation        | ↑                 | ↑            | [5]       |
| Amylase            | In vitro-translation        | ↓                 | ↑            | [5]       |
|                    | cDNA hybridization          | ↓                 | ↑            | [24]      |
|                    |                             |                   | ↑            | [10]      |
| Procarboxypeptidase| In vitro-translation        | →                 | →            | [5]       |
|                    | cDNA hybridization          | →                 | →            | [6]       |
study measuring mRNAs coding for proelastase, chymotrypsinogen, trypsinogen and amylase, it was noted that proelastase mRNAs did not follow the other serine proteases and amylase by staying fairly unaffected by protein-deprivation for five days [9]. Despite the upregulation of the serine-protease and PSTI mRNAs, it has to be kept in mind that overall protein synthesis and secretion is reduced in rats fed a protein-free diet (see below). Thus, the synthesis of serine proteases appears to be regulated at the transcriptional as well as translational level.

**POTENTIAL FOR PROTEIN SYNTHESIS IN PANCREATIC ACINAR LOBULES**

Several studies with the aim to understand regulatory mechanisms underlying the pancreatic response to dietary adaptations have been performed (summarized in Table 2). Adaptation to a diet was estimated based on the rate of synthesis of individual proteins in pancreatic lobules isolated from rats after a 12-day administration of different diets. As a general rule, (pro)enzyme synthesis was proportional to the respective substrate content, i.e., proteases were synthesized predominantly after feeding a protein-rich diet while amylase synthetic rate was coupled to a high dietary carbohydrate content.

The rate of synthesis of enzymes after the most extreme diet, the zero protein/high carbohydrate diet, took an unexpected turn [4]. Biosynthetic rates for serine proteases (trypsinogen, chymotrypsinogen, procarboxypeptidase) increased significantly compared to their levels after a normal diet, while the synthetic rate decreased for amylase to a level similar to the one reached with a zero carbohydrate/high protein diet. But there was another even more surprising observation: When individual isoforms of (pro)enzymes were studied separately, synthesis of anionic isoforms increased to levels similar or higher than their corresponding levels at a high protein diet, whereas synthesis of cationic or neutral isoforms was reduced. After a 12-day administration of a protein-free diet, synthesis of anionic proteins in pancreatic lobules went up from 45.6 percent of total protein synthesis (under normal dietary conditions) to 95 percent. This interesting correlation between the regulation of individual isoforms and their respective isoelectric points under conditions of a low protein diet may reflect an adaptive response crucial to cell survival in a protein-deficient environment. The continued synthesis and secretion of a select group of proteases might enable the organism to digest newly found sources of protein.

Since fractional synthetic rates reflect the relative distribution of newly synthesized proteins, additional criteria for regulation of secretory enzymes have to be kept in mind: first, this type of measurement does not take into account the absolute synthetic potential of the cells. Thus, it is possible that a shift towards anionic forms of enzymes occurs along with a reduction of the synthetic output. Indeed, when tissue content of activatable (pro)enzymes were determined, a significant reduction in protease activity was observed, particularly, in the protein-deficient diet [4, 10]. This observation was supported by an electron microscopical determination of zymogen granule density which was vastly decreased in acini of rats treated with the protein-free diet compared with the control diet [4].

In a separate study [11], tissue contents of amylases, trypsinogen(a), chymotrypsinogen(a) and lipase were determined by FPLC separation of pancreatic zymogen granule proteins. Here, accumulations of both proteases in the pancreas were proportional to the dietary protein fed for ten days. In contrast, lipase and amylases were regulated independently of the dietary protein, but were synthesized according to their respective primary substrates.

We conclude that the levels of enzymes produced are in agreement with Pavlov’s hypothesis of a substrate-dependent induction of enzyme synthesis. Furthermore, the shift
Table 2. Adaptation of protein levels in the rat pancreas as a response to various diets. One diet was rich in protein (82 percent) [4] and the others were protein deficient diets (0 percent protein content) [4], (2 percent protein content) [10]. Arrows indicate upregulation (↑), downregulation (↓), slight but not significant down regulation (\(\geq\)) or no change (\(\rightarrow\)) relative to a control diet containing 22 percent protein [4] or 18 percent [10]. Rats were fed the experimental diet for 12 days [4] or four weeks [10]. Data from other sources were not included because they represented short-term adaptations [25] of pancreatic proteins from protein-rich to carbohydrate-rich diets and vice versa or used dietary schemes [11] that were not compatible with the overview in this table. Fractional synthetic rates are calculated as percent incorporation of radioactive label in a given protein compared to the total incorporation of label in all proteins combined.

| Protein                        | Method                          | Protein deficient | Protein rich | Reference |
|--------------------------------|--------------------------------|------------------|--------------|-----------|
| Trypsinogen anionic (1+2)      | Fractional synthetic rates      | ↑                | ↑            | [4]       |
| Trypsinogen cationic           | Fractional synthetic rates      | ↓                | →           | ibid      |
| Chymotrypsinogen anionic       | Fractional synthetic rates      | ↑                | ↑            | ibid      |
| Chymotrypsinogen cationic      | Fractional synthetic rates      | ↓                | ↑            | ibid      |
| Proelastase anionic            | Fractional synthetic rates      | ↑                | ↑            | ibid      |
| Proelastase cationic           | Fractional synthetic rates      | ↓                | ↓            | ibid      |
| Amylase (1+2)                  | Fractional synthetic rates      | ↓                | ↓            | ibid      |
| Procarboxypeptidase (A+B)      | Fractional synthetic rates      | ↑                | ↑            | ibid      |
| Trypsin(ogen)                  | Tissue content                  | \(\geq\)         | ↑            | [4]       |
|                               |                                 | \(\rightarrow\)  |              | [10]      |
| Chymotrypsin (ogen)            | Tissue content                  | ↓                | ↑            | [4]       |
|                               |                                 | ↓                |              | [10]      |
| Amylase                        | Tissue content                  | ↓                | ↓            | [4]       |
|                               |                                 | ↓                |              | [10]      |
| Protein synthesis              | Incorporation of amino acids    | ↓                |              | [26]      |

in the relative synthetic potential towards those enzymes that are deprived of dietary substrate (e.g., anionic serine proteases in the zero protein diet) may reflect an "anticipation" of the acinar cells to a rise of protein content in the diet. Alternatively, it might be speculated that the anionic forms are better suited to autodigest the organism's own tissue under starving conditions [12]. It is also conceivable that these anionic forms may digest insoluble/inaccessible proteins better than cationic forms or may be more stable in an duodenal environment lacking proteins.
HORMONAL REGULATION OF PROTEIN SYNTHESIS

Under physiological conditions, the pancreas is regulated not only by changes in the nutritional substrates, but by the influence of hormones and neurotransmitters onto the acinar cells. Changes in enzyme synthesis due to cholecystokinin (CCK) and CCK-like hormones [13] are different for different isoforms, too. They are similar to those observed after administration of a high protein diet, suggesting that CCK may mediate the effects of protein ingestion. The release of CCK depends on the presence of free monitor peptide, which is identical to the pancreatic secretory trypsin inhibitor form 1 (PSTI-I) [14]. This molecule binds to trypsin and dissociates in the presence of protein substrate (food) in the duodenum. Once released it stimulates endocrine cells to secrete CCK [14]. Thus, it is assumed that at a high duodenal protein content, monitor peptide is totally dissociated from trypsin, free to induce CCK. After ingestion of a protein-free diet, however, monitor peptide is tightly complexed with trypsin and therefore not capable of stimulating a CCK-release.

Regulation of synthesis of other classes of (pro)enzymes has been correlated with their respective substrates and the circulation of hormones: indirect evidence suggests that amylase synthesis appears to be under the control of insulin [15-17] after starch uptake, while lipase synthesis is promoted by the consumption of fatty acids and has been shown to be under the control of secretin [18].

GENETIC APPROACHES TO SUBSTRATE-INDUCED GENE EXPRESSION

A meticulous analysis of the regulation of the elastase gene has been reported by MacDonald and coworkers. They have shown that the elastase enhancer consists of a tripartite (A,B,C) regulatory element [19] preceding the TATA-box. A sequence as short as 134 bp (from -205 to -72) is sufficient to direct expression of the gene at the correct developmental time and in a tissue- and cell-specific manner [20]. Other genes have been studied or its putative promotor/enhancer sequences compared to the elastase enhancer. In genes coding for other proteases (i.e., chymotrypsinogen B, elastase II, trypsin I and trypsin II), there appears to be a sequence conservation homologous to the A-element in the elastase enhancer. However, the overall structure of the promotor region in the trypsin I gene appears to differ from the elastase promotor. Analysis of promotors have mainly dealt with tissue specificity and levels of expression but not with experimental substrate induction by proteins. The analysis of the amylase promotor, however, has demonstrated, besides tissue and cell specificity, elements responsive to cellular stimulation by insulin and diet [17, 21]. Analysis of promotor/enhancer elements in conjunction with the determination of transcript levels in transgenic mice is still a powerful tool to understand the complex mechanisms of gene expression. With the possibility to generate transgenic rats [22], an even more interesting tool is available since many studies involving dietary control of protein synthesis and secretion have been performed in this species.

CONCLUDING REMARKS

In this brief outline we have not discussed the effect of hormones on the fractional and absolute synthetic rates after exposing rats to the diets. Rather, it reflects the basal status of the acinar cell after treatment with various diets. Furthermore, control of individual (pro)enzyme synthesis by hormones is not directly coupled to hormonal regulation of secretion of these proteins. As an example, CCK is responsible for secretion of most pancreatic enzymes but promotes the synthesis selectively of the proteases but not the lipases or amylase. Thus, a further distinction between diet-induced protein synthesis and its secretion has to be made.
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