Pancreatic Islet–Acinar Cell Interaction: Amylase Messenger RNA Levels Are Determined by Insulin

Abstract. *Pancreatic amylase messenger RNA progressively decreases in rats rendered diabetic with streptozotocin. Insulin reverses this effect, inducing a selective increase in amylase messenger RNA in the pancreas. Parotid amylase messenger RNA is not significantly affected by either diabetes or insulin.*

The pancreas of higher vertebrates exhibits a complex cellular organization in which clusters of several types of endocrine cells are dispersed throughout the exocrine organ. While the reasons for this unusual anatomic arrangement are unclear, the proximity of endocrine elements to the enzyme secreting acinar tissue has given rise to the hypothesis that islet-cell hormones may modulate pancreatic exocrine activity (*1–3*). We now report that insulin has a decisive influence on pancreatic amylase messenger RNA (mRNA), and provide functional evidence on a molecular level for a specific endocrine-acinar cellular interaction. In contrast to insulin's more modest effects on a number of cellular metabolic enzymes, the magnitude of the insulin effect on both pancreatic amylase protein and mRNA levels is large. As a consequence of this regulation, there may be a substantial reduction in amylase in the gut of insulin-deficient organisms; the resultant decrease in carbohydrate absorption and glucose utilization from starch may therefore have a protective effect. In this sense, insulin's inductive effect on amylase may represent yet another mechanism whereby this hormone regulates glucose homeostasis.

To determine the consequence of insulin deficiency on pancreatic enzyme content, we measured pancreatic amylase and chymotrypsinogen activities in both normal and diabetic rats. Rats (100 to 130 g) were injected intravenously with streptozotocin, according to regimens that routinely cause a 20-fold decrease in serum insulin, and a fourfold increase in serum glucose (*4*). This treatment resulted in an 11-fold decrease in pancreatic amylase after 6 days of streptozotocin diabetes and a further 32-fold decrease by day 13 (Table 1). In contrast to this decrease (300-fold) in pancreatic amylase content, the pancreatic chymotrypsinogen content increased slightly (Table 1).

Our findings agree with those of previous studies in which alloxan-induced diabetes also resulted in a decrease in pancreatic amylase and a rise in chymotrypsinogen (*5, 6*). In these and other studies with streptozotocin as the B cell toxin (*7, 8*), insulin reversed the fall in pancreatic amylase. Other types of experiments also suggest that insulin may regulate pancreatic amylase content: Both insulin-dependent diabetes in humans (*9, 10*) and spontaneous diabetes in animals (*11*) are associated with decreased amylase secretion from the exocrine pancreas, and the severity of this dysfunction correlates directly with B cell secretory malfunction (*12*); excessive insulin secretion leads to increases in pancreatic amylase in normal animals (*13*); insulin, in vitro, increases protein synthesis and amylase content in isolated pancreatic acini prepared from diabetic rats (*4*).

The availability of cloned amylase complementary DNA (cDNA) (*14*) and cloned chymotrypsinogen cDNA (*15*) allows a disciminating quantitative assay for the respective mRNA species. The levels of pancreatic mRNA for amylase and chymotrypsinogen were therefore determined by hybridization techniques with these cDNA's. There was an 11-fold decrease in pancreatic amylase mRNA after 6 days of diabetes (Table 1 and Fig. 1). A further decrease to undetectable levels occurred after 13 days. Thus, there was a 100-fold decrease in amylase mRNA in these animals as compared to control animals (legend to Table 1). Along with this decrease in amylase mRNA, there was a slight increase in chymotrypsinogen mRNA (Table 1 and Fig. 1). This indicated that both amylase and chymotrypsinogen mRNA's changed in parallel with their respective protein levels. Since streptozotocin-induced diabetes results in only a slight decrease in parotid amylase (*8*), both parotid amylase protein and mRNA were also measured. In contrast to the changes observed in the pancreas, there was only a slight decrease in parotid amylase content, and no significant

Table 1. Effect of diabetes and insulin on protein and mRNA levels for amylase and chymotrypsinogen. Male Sprague-Dawley rats (100 to 130 g) were injected intravenously with either streptozotocin (75 μg/g) or 100 m*M* citrate buffer, *p*H 4.3 (*4*). Six days later, three diabetic (D6) and three control (N) animals were then killed. The remaining diabetic animals were subdivided into two groups: one received 4 units of Lente insulin per 100 g of body weight, and the other was untreated. After seven daily injections of insulin, three insulin-treated rats (D617), three diabetic rats (D13), and three additional control rats (N) were killed; the pancreatic and parotid tissues of these animals were rapidly removed, and fragments (approximately 25 mg, wet weight) were cut and frozen for enzymatic assay. To prepare RNA, the remaining tissues were rapidly homogenized in 8 ml of 4*M* guanidine thiocyanate, 100 m*M* tris-HCl (*p*H 7.5), and 2*M* β-mercaptoethanol (*19*). Homogenates from either three individual pancreases or parotid glands were pooled and layered over 5.7*M* CsCl containing 0.1*M* sodium EDTA (*p*H 7.0) and centrifuged at 36,000 rev/min (SW41 rotor, Beckman) for 21 hours at 20°C. The RNA pellet was dissolved in water treated with diethylpyrocarbonate (Sigma). Messenger RNA was quantified by covalently binding 1.0 μg of total unfractionated RNA to diazobenzyloxmethyl (DBM) paper (Schleicher and Schuell) (*20*). The paper containing RNA was then hybridized with either ^{32}P-labeled amylase or chymotrypsinogen cDNA probes. The amylase probe was derived from plasmid clones pcXP-38 and pcXP-100 (*14*), and chymotrypsinogen probe was derived from plasmid clones pcXP-33 and pcXP-35 (*15*). Probes were labeled to specific activities of approximately 10^8 cpm/μg DNA by nick translation (*21*). After hybridization, DBM filters were washed to remove nonspecifically bound label; details of hybridization procedures are described in the legend for Fig. 1. The areas containing the radioactive spots (2 cm^2) were cut out, dried and counted with Omnifluor scintillation fluid (New England Nuclear). Contiguous areas of filter not containing bound RNA were counted to determine nonspecific hybridization. Values are the mean ± standard deviation (S.D.) of triplicate determinations after subtraction of background. Amylase was assayed by the method of Rinderknecht with amylose azure as substrate (*22*), and chymotrypsinogen was assayed by the method Erlanger with *N*-glutaryl-L-phenyalanine *p*-nitroanilide as substrate (*23*). Values are the means ± S.D. of duplicate determinations from each of three individual pancreases or parotid glands.

Group	Amylase		Chymotrypsinogen	
	Activity	mRNA (cpm)	Activity (μmole/min per milligram of protein)	mRNA (cpm)
N	7.23 ± 1.14	1240 ± 300	24.4 ± 2.8	210 ± 30
D6	0.64 ± 0.29	110 ± 20	34.4 ± 7.3	250 ± 40
D13	0.02 ± 0.01	< 15*	30.3 ± 2.9	340 ± 80
D617	5.77 ± 2.75	580 ± 110	15.8 ± 8.5	150 ± 30
N	7.92 ± 0.50	870 ± 130		
D13	5.37 ± 0.75	910 ± 150		
D617	10.34 ± 0.43	530 ± 110		

*Signals could be detected by autoradiography at 1 percent of normal amylase mRNA concentrations; no signal was detected.

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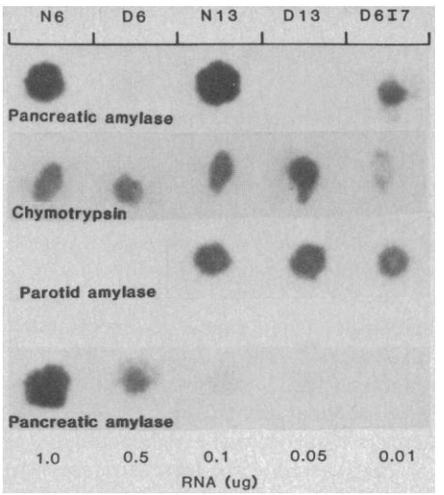

Fig. 1. Effects of diabetes and insulin on amylase and chymotrypsinogen mRNA. RNA was isolated and covalently bound to DBM paper as described in Table 1. All samples to be hybridized with a specific probe were bound to a single sheet of DBM paper to avoid interassay variation. The paper was treated (at 42°C for 15 to 24 hours) with a mixture of 5 × SSC (1 × SSC is 0.15*M* NaCl, 0.15*M* sodium citrate), 5 × Denhardt's solution (*24*), 50 m*M* sodium phosphate buffer (*p*H 6.5), 50 percent deionized formamide, and sonicated denatured salmon sperm DNA (500 μg/ml). Filters were then hybridized overnight with the above prehybridization solution made to 10 percent with dextran sulfate 500 (*25*) and heat denatured ^{32}P-labeled amylase or chymotrypsinogen probe (5 × 10^5 cpm/ml). Filters were washed in 0.1 × SSC, 0.1 percent sodium dodecyl sulfate at 50°C and exposed to x-ray film (Kodak XR-2) with intensifying screen (DuPont Lightning-Plus) at −70°C for 7 to 24 hours. The symbols N6, D6, N13, D13, and D6I7 represent normal 6 days, diabetic 6 days, normal 13 days, diabetic 13 days, and diabetic 6 days plus insulin 7 days, respectively (see Table 1). Dilutions of pancreatic RNA from control rats were also spotted onto DBM paper at 1.0, 0.5, 0.1, 0.05, and 0.01 μg of total RNA to determine the minimum levels at which amylase mRNA could be detected; we had previously determined that our assay was linear to 3.0 μg/cm^2. Specific hybridization with amylase probe was detected with 0.01 μg of RNA (a spot is visible in the original autoradiogram).

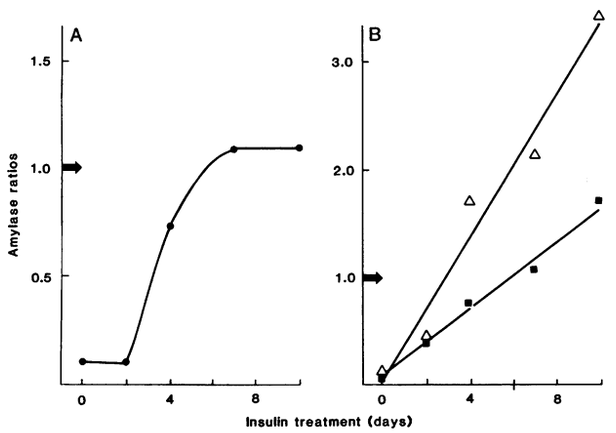

Fig. 2. Time course of insulin action on amylase protein levels, and amylase, chymotrypsinogen, and elastase mRNA. Rats were made diabetic with streptozotocin, and 6 days later insulin was given daily for 10 days (see Fig. 1). The control animals were killed on day 6 of diabetes (no insulin treatment), and the others were divided into groups, one group being killed on each of days 2, 4, 7, and 10 of the insulin treatment. (A) Pancreatic amylase activity was assayed as described in Table 1. Values are normalized to amylase levels in control rats (indicated by arrow). (B) The relative amounts of amylase, chymotrypsinogen, and elastase mRNA were determined by examining the ratios of amylase to chymotrypsinogen (△) and amylase to elastase (■). Values are normalized to the mRNA ratios of amylase to chymotrypsinogen and amylase to elastase in control rats (indicated by arrow), and fitted by the method of least squares. Elastase probe was prepared from plasmid clone pcXP-13 (*26*).

Table 2. Time course of insulin effects on pancreatic mRNA. Rats were made diabetic and 6 days later were either untreated or injected daily with insulin (Fig. 2). Animals were divided into groups; the untreated group animals were killed before insulin treatment and groups 2 (D612), 4 (D6I4), 7 (D6I7), and 10 (D6I10) were killed after 2, 4, 7, and 10 days, respectively, of insulin treatment. Total pancreatic RNA was isolated, and 1.0-μg samples were spotted onto DBM paper. The RNA was then hybridized with amylase, chymotrypsinogen, and elastase probes. Areas surrounding each radioactive spot (2 cm^2) were cut out and counted. Values are the means ± standard deviation of triplicate determinations, after subtracting background.

Group	Treatment time (days)	mRNA (cpm)		
		Amylase	Chymotrypsinogen	Elastase
Control		1200 ± 190	590 ± 180	730 ± 200
Diabetic nontreated				
D6		280 ± 40	1310 ± 180	1300 ± 110
D10		<50*	712 ± 175	1103 ± 380
Diabetic treated				
D6I2	2	650 ± 40	780 ± 110	980 ± 140
D6I4	4	790 ± 10	230 ± 50	630 ± 70
D6I7	7	1950 ± 10	450 ± 110	1120 ± 170
D6I10	10	7040 ± 340	1000 ± 20	2500 ± 210

*A very faint spot was visible by autoradiography after long exposure to x-ray film, but accurate quantitations could not be performed.

change in parotid amylase mRNA (Table 1 and Fig. 1).

To determine whether insulin reverses the diabetes-induced decrease in pancreatic amylase mRNA, we administered insulin to rats that had been rendered diabetic 6 days previously. After the animals were treated with insulin for 7 days, both pancreatic amylase protein and mRNA levels increased (Table 1 and Fig. 1). In contrast, there was a slight concomitant decrease in both chymotrypsinogen protein and mRNA (Table 1 and Fig. 1). In the parotid gland, insulin caused only a slight increase in amylase protein, and no significant change in amylase mRNA (Table 1 and Fig. 1).

Pancreatic amylase mRNA was significantly increased after 2 days of insulin treatment and returned to normal within 7 days. After 10 days of insulin treatment amylase mRNA was above normal (Table 2), and was 25-fold higher than that in rats with induced diabetes at day 6. This increase was considerably greater in relation to rats that were diabetic for 10 days. In contrast, pancreatic amylase protein did not increase until day 4, and reached a maximum after day 7 of insulin treatment (Fig. 2A). Although chymotrypsinogen and elastase mRNA were somewhat variable and less dramatically affected by insulin (Table 2), the ratios of amylase mRNA to either chymotrypsinogen or elastase mRNA increased linearly throughout the insulin treatment. This implies that the variations in chymotrypsinogen and elastase mRNA were not random. In addition, the slope of the line determined by the ratios of the amylase mRNA to chymotrypsinogen mRNA was twice the slope of the line determined by the ratio of amylase mRNA to elastase mRNA (Fig. 2B). This difference indicates that the effect of insulin may not be restricted to amylase. It is possible, therefore, that insulin has an inverse effect on amylase and chymotrypsinogen mRNA. Such opposite actions could either be caused directly by insulin, or could be due to competition of the genes for a limited number of RNA polymerase molecules.

Our experiments demonstrate a selective and dramatic effect by insulin on amylase gene expression. This specific action is not the result of a general defect in pancreatic protein synthesis, since chymotrypsinogen was increased in diabetes. While insulin also selectively induces both the activity of certain enzymes and the synthesis of specific proteins in various cells, these effects are not of the same magnitude as in our study. In the developing rat brain, for example, insulin causes a five- to eightfold increase in ornithine decarboxylase

activity (*16*). In the liver of diabetic rats, insulin causes a sixfold increase in glycogen phosphatase activity (*17*), a twofold increase in glycogen synthase activity (*17*), and a fivefold increase in albumin synthesis (*18*). The latter effect is accompanied by a threefold increase in albumin mRNA (*18*). Our data therefore demonstrate the most pronounced inductive effect by insulin on a specific gene product. It is not known, however, whether insulin changes pancreatic amylase mRNA by stimulating its synthesis or inhibiting its degradation. The apparently long lag period required for this inductive effect raises the question whether insulin acts directly or indirectly an amylase gene transcription. Studies on cellular systems, in vitro, may provide conclusive answers to these questions. Because of the dramatic effects on a major gene product, the pancreatic acinar cell may be a useful model system for studying the molecular actions of insulin.

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Tonic Immobility Produces Hyperalgesia and Antagonizes Morphine Analgesia

Abstract. *Hyperalgesia was demonstrated during and immediately after termination of tonic immobility in the lizard* Anolis carolinensis. *Additionally, tonic immobility antagonized morphine-induced analgesia. In conjunction with other research, these data suggest that the response is accompanied by a reduced availability of serotonin, possibly at postsynaptic receptors of raphe neurons.*

Tonic immobility (TI) is a catatonic-like state of behavioral inhibition elicited by brief physical restraint exhibited in a wide variety of species including some mammals, fowl, reptiles, fish, and insects (*1*). In a typical laboratory setting, an animal restrained on its side or back will cease struggling after a few seconds. After restraint is removed, the animal will remain immobile for periods varying from a few seconds to well over an hour. The response is characterized by inhibition of movement, waxy flexibility, intermittent eye closures, and Parkinsonian-like tremors. Tonic immobility is very sensitive to manipulations involving fear, and it has been proposed as a predator defense mechanism of last resort (*1*). Gallup and Maser (*2*) have suggested a possible connection between TI and human catalepsy and catatonia.

Of primary interest to researchers are the neural mechanisms underlying TI. The model most strongly supported has been the serotonergic-midbrain raphe model proposed by Wallnau and Gallup (*3*). The focus of this model is the inverse relationship between drug-induced effects on TI durations and their effects on activity of the raphe nuclei. Presumably, administration of serotonin (5HT) or its agonists results in excess 5HT at postsynaptic receptors producing inhibitory feedback to the raphe, which in turn produces concomitant increases in TI durations. In light of this model, the apparent relationship between 5HT, the raphe, and nociception (*4, 5*) makes the nature of pain sensitivity during TI an interesting (yet essentially unanswered) question. Enhancing the significance of this question is the possibility that endogenous release of opiate peptides (endorphins and enkephalins) may be involved in TI mechanisms (*6*). Despite the theoretical appeal of such information, researchers have previously found quantification of nociception difficult in animals that exhibit the TI response.

We now report the lizard *Anolis carolinensis* to be an excellent preparation for the objective study of pain sensitivity accompanying TI. We found the anole lizard, which has previously been used in TI research (*7, 8*), well suited for tests in a tail-flick apparatus. Tail-flick latencies for lizards tested 15 seconds after induction of TI with their tails positioned in the apparatus were compared with those tested after comparable handling. The TI-treated lizards demonstrated increased sensitivity to noxious stimuli as evidenced by significantly shorter tail-flick latencies [1.667 and 2.212 seconds, respectively; $F(1, 28) = 11.38$, $P < .005$]. Control lizards were also compared with those tested immediately after either 15 or 30 seconds of TI, which was terminated by the experimenter. Again, TI-treated lizards exhibited shorter tail-flick latencies [$F(2, 57) = 4.686$, $P < .02$]. Thus, lizards were more sensitive (*9*) to noxious stimuli during and immediately after TI was externally terminated. An earlier, subjective report of analgesia during TI in rabbits (*10*) suggests that these effects may not generalize to that species. The subjective nature of this report leaves the question of generalization of TI effects to rabbits relatively unresolved (*11*).

The TI-induced changes in nociception reported here are quite similar to effects produced by depletion of 5HT (*7*). *para*-Chlorophenylalanine depletion of 5HT or lesions of the 5HT-rich raphe nuclei produce hyperalgesia as well as antagonism of morphine analgesia (*5*). The similarities between these independent findings and our data suggest that

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