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[SHORT COMMUNICATON]

Glucose-stimulated Somatostatin Gene Expression in the Brockmann Bodies of Rainbow Trout (*Oncorhynchus mykiss*) Results from Increased mRNA Transcription and not from Altered mRNA Stability

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ABSTRACT—Previously, we showed that glucose increases the steady-state levels of the mRNAs encoding two distinct preprosomatostatins (each containing [Tyr^7, Gly^{10}]-somatostatin-14 at their C-termini; denoted PPSS II' and PPSS II") in the endocrine pancreas (Brockmann body) of rainbow trout (*Oncorhynchus mykiss*). In the present study, isolated islet cells were used to determine whether glucose-stimulated expression resulted from altered rates of transcription and/or from changes in RNA stability. Nuclear run-on assays indicated that the number of PPSS II nascent transcripts were significantly higher in nuclei isolated from islet cells cultured in 10 mM glucose compared to those isolated from cells incubated in 4 mM glucose. High glucose (10 mM) did not, however, affect the stability of PPSS II mRNAs. These results indicate that glucose-stimulated somatostatin expression in the Brockmann bodies of rainbow trout results from increased endogenous mRNA transcription and not from altered mRNA stability.

Key words: somatostatin, glucose, transcriptional regulation, rainbow trout

INTRODUCTION

Somatostatins (SSs) are a multi-functional family of peptide hormones that coordinate a vast array of physiological processes, from modulation of growth and differentiation to regulation of metabolism (Patel, 1999; Tannenbaum and Epelbaum, 1999). Many SS variants, differing in both amino acid chain length and amino acid composition, have been isolated and all vertebrates studied to date, from agnathans to mammals, possess one or more forms of SS (Conlon *et al*., 1997). The structural heterogeneity of the SS family stems from the differential processing of the hormone precursor and/or from the existence of multiple SS genes (Sheridan *et al*., 2000). Mammals possess SS-14, the first SS sequence characterized (Brazeau *et al*., 1973), in addition to a number of N-terminally extended forms (*e.g*., SS-28)(Conlon *et al*., 1997); these various forms arise from the tissue-specific processing of a single large precursor known as preprosomatostatin I (PPSS I)(Conlon, 1989).

Lamprey, teleost fish, and frogs possess PPSSs in addition to PPSS I; these various precursors appear to derive from different genes (Conlon *et al*., 1997). For example, salmonids possess SS-14 as well as at least one other more abundant peptide which contains [Tyr^7, Gly^{10}]- SS-14 at its C-terminus, salmonid SS-25 (Plisetskaya *et al*., 1986). We recently showed that rainbow trout express three distinct mRNAs: one encoding a PPSS I that contains SS-14 at its C- terminus (Kittilson *et al*., 1999) and two encoding PPSSs that contain [Tyr^7, Gly^{10}]-SS-14 at their C-termini, denoted PPSS II' and PPSS II"(Moore *et al*., 1995, 1999). Increasing evidence suggests that there are functional differences among the various SS forms (Sheridan *et al*., 2000).

In this study, we used rainbow trout (*Oncorhynchus mykiss*) to evaluate further the gene expression of SSs. Previous work in our laboratory revealed that PPSS II' and PPSS II" mRNAs in the Brockmann bodies of rainbow trout are differentially expressed (Moore *et al*., 1999) and that glu-

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cose regulates the pattern of their expression (Ehrman *et al.*, 2000). The present study was designed to reveal the mechanism(s) through which glucose modulates the expression of these two distinct mRNAs in the Brockmann bodies of rainbow trout.

MATERIALS AND METHODS

Animals

Juvenile rainbow trout (*Oncorhynchus mykiss*) of both sexes were obtained from Dakota Trout Ranch near Carrington, ND, USA and transported to North Dakota State University where they were maintained in 800 L circular tanks supplied with recirculated (10% make-up volume per day) dechlorinated municipal water at 14°C under a 12:12hr, light-dark photoperiod. Fish were fed to satiety twice daily with Supersweet Feeds (Glenco, MN, USA) trout grower, except 24–36 hr before experiments. Animals were acclimated to laboratory conditions for at least 4 weeks prior to experiments. On the day of experiments, fish were anesthetized in 0.5% (v/v) 2- phenoxyethanol and their Brockmann bodies were removed and prepared for analyses as described below.

Experimental conditions and analyses

The transcription of PPSS II mRNAs was assessed by quantification of nascent transcripts in nuclei isolated from islet cells. Brockmann bodies were placed in Puck's medium (in nM: 137 NaCl, 5.4 KCl, 4 $NaHCO_3$, and 4 glucose) and cut into ca. $1mm^3$ pieces. The pieces were incubated for 1 min in Puck's containing 5mM EDTA and then rinsed four times in Puck's. Islet cells were dispersed from tissue pieces incubated at room temperature under continuous gentle stirring in Puck's containing 2.0 U/mL dispase II (Boehringer Mannheim) and 1.0 μg/mL DNase. The cells were collected by centrifugation (100 × g for 5 min at 14°C), washed twice by centrifugation/resuspension with DMEM [in mM: 137 NaCl, 5.4 KCl, 4 $NaHCO_3$, 1.7 $CaCl_2$, 0.8 $MgSO_4$, 0.5 KH_2PO_4, 0.3 Na_2HPO_4, 10 HEPES, 4 glucose, 4 glutamine with 5% (v/v) fetal calf serum and 1× antibiotic/antimycotic solution (Sigma A 9909), pH 7.6], and then incubated overnight at 14°C in 25-ml flasks containing DMEM. After "recovery," the cells were collected and resuspended in DMEM containing either 4 mM glucose or 10 mM glucose and incubated (14°C, 100% O_2, shaken at 100 rpm with a gyratory shaker) for up to12 hr. These concentrations of glucose were selected to mimic basal (4 mM) and high glucose (maximum stimulation of PPSS II mRNA) conditions. Nuclei isolation (from ca. 1×10^7 cells/sample) and nuclear run-on assays were performed as described previously (Greenberg, 1994). Labeled nuclear transcripts were hybridized to specific PPSS cDNA probes previously immobilized on nylon membrane by use of a slot-blot apparatus. Blots were quantitated by phosphor imaging as described previously (Ehrman *et al.*, 2000).

The stability of PPSS II mRNAs also was determined in isolated islet cells. Following recovery, islet cells were centrifuged, washed, and resuspended in fresh DMEM with 5 μg/ml actinomycin D. After 30 min, the cells were collected and resuspended in DMEM containing 4 mM glucose or 10 mM glucose (NaCl concentration adjusted to be isosmotic). At various times after this medium change, the cells were collected and their total RNA were extracted and analyzed as described previously (Ehrman *et al.*, 2000).

Statistical differences were estimated by analysis of variance; multiple comparisons among means were made with the Student-Newman-Keul's test. Differences were considered significant at $P < 0.05$. For ease of comparison, data were expressed as% change (final level- initial level/initial level × 100); statistics were performed on untransformed data.

RESULTS AND DISCUSSION

This study was designed to determine if glucose-stimulated increases in steady state levels of PPSS mRNAs (Ehrman *et al.*, 2000) result from increased rates of PPSS mRNA transcription and/or from changes in the stability of PPSS mRNAs.

Nuclear run-on assays were performed to determine whether transcription of the endogenous PPSS genes is involved with glucose-stimulated PPSS II mRNA levels. With this approach, the rate of formation of nascent PPSS II transcripts was used to determine the effects of glucose on PPSS gene transcription. The resulting labeled nuclear transcripts from cells incubated in 4 mM glucose and 10 mM glucose were hybridized to trout PPSS II cDNAs immobilized on nylon membranes (insets, Fig. 1). Glucose, at a concentration of 10 mM, significantly increased the transcription rates of both PPSS II' and PPSS II" compared to rates in islet cells incubated in 4 mM glucose (Fig. 1), an effect that was apparent after 6hr. Notably, the effect of glucose was more pronounced on PPSS II" transcription than on PPSS II' transcription after 12 hr.

The possibility that the stability of PPSS mRNAs underlies glucose-stimulated steady state levels of PPSS II mRNAs also was examined. The half-life of PPSS II mRNAs was compared in cells incubated in 4 mM (control) and 10 mM glucose (Fig. 2). The rates of PPSS II' and PPSS II" mRNA degradation were similarly rapid in cells incubated in 4 mM glucose. High glucose (10 mM), however, had no effect on the stability of PPSS II mRNAs.

Glucose-stimulated expression of PPSS II mRNAs results from increased transcription of endogenous PPSS II genes and not from altered PPSS II mRNA stability. This conclusion is supported by several observations. First, previous experiments showed that glucose-stimulated expression of PPSS II' and PPSS II" mRNAs could be blocked by actinomycin D (Ehrman *et al.*, 2000). Second, nuclear run-on assays from the current study indicated that the number of PPSS II nascent transcripts were significantly higher in nuclei isolated from islet cells cultured in 10 mM glucose compared to those isolated from cells incubated in 4 mM glucose. Notably, glucose-stimulated PPSS II mRNA transcription paralleled glucose-induced increases in the steady state levels of PPSS II mRNAs. Lastly, the inability of glucose to alter PPSS II mRNA degradation supports the notion that glucose-stimulated expression of PPSS II mRNAs does not involve alterations in PPSS II mRNA stability.

The exact means by which glucose activates transcription of PPSS II genes remains to be determined. Our previous study indicated that glucose metabolites generated after the aldolase step of glycolysis were capable of stimulating expression of PPSS II mRNAs (Ehrman *et al.*, 2000). It is conceivable that one or more of these metabolites could activate specific *trans*-acting factors that, in turn, modulate gene transcription via carbohydrate-responsive elements (CHORE) in promoter regions of the SS genes, as is the

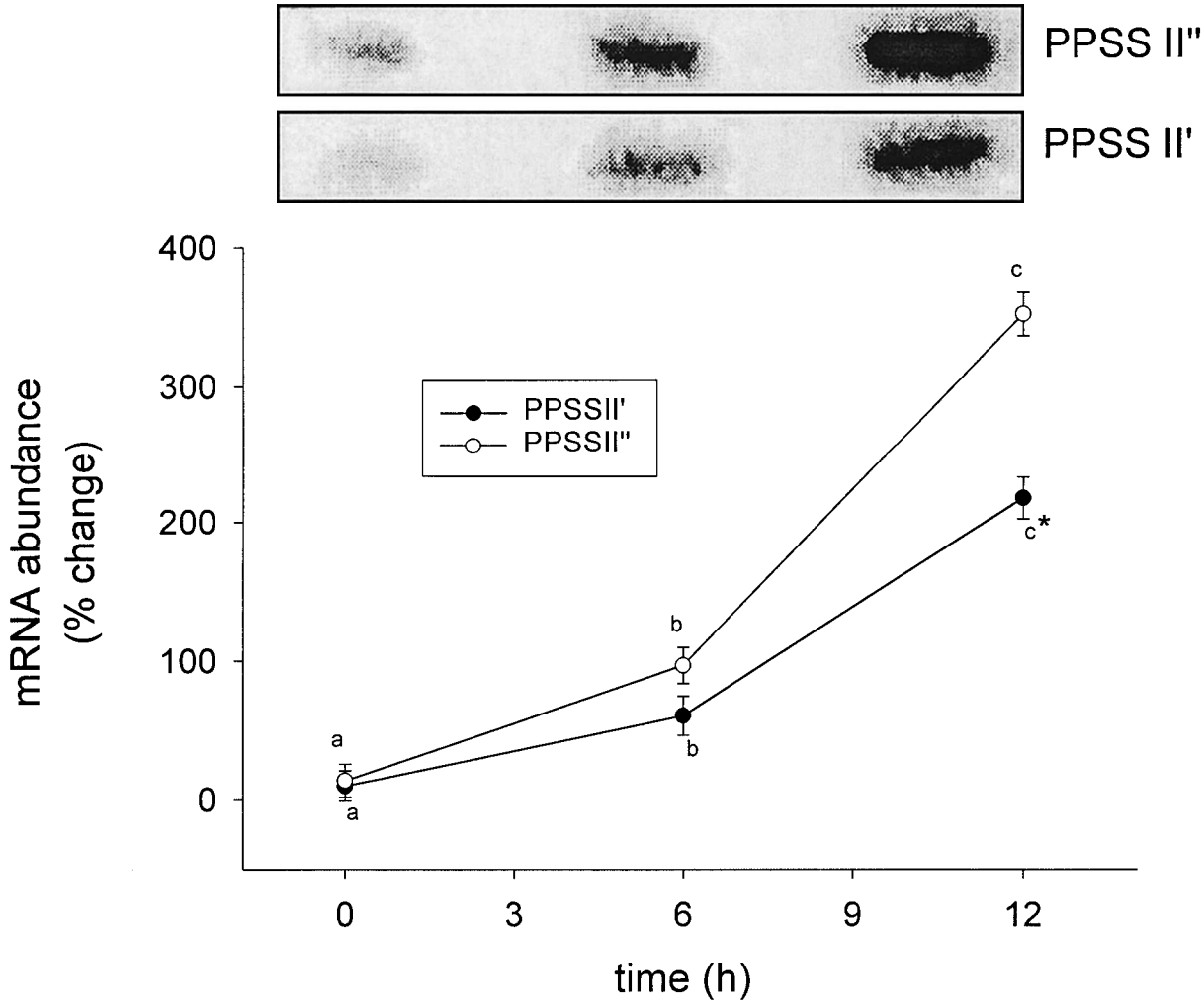

Fig. 1. Glucose stimulates transcription of preprosomatostatin (PPSS) mRNAs. Nuclear run-on transcription assays were performed on nuclei isolated from dispersed rainbow trout islet cells incubated in the presence of 10 mM or 4 mM (control) glucose for the times indicated as described in Materials and Methods. Labeled nuclear transcripts were hybridized to specific PPSS cDNA probes immobilized on nylon membranes (representatives shown in inset). Blots were quantitated by phosphor imaging and the data expressed as percent change (means±S.E.M., n=6) from control. For a given RNA species, groups with different letters are significantly ($p < 0.05$) different form one another. * indicates significant difference from PPSS II' counterpart group.

case for other glucose sensitive genes (*e.g.*, S14; Sudo and Mariash, 1994).

The finding that glucose stimulates the expression of PPSS II mRNAs via transcriptional regulation extends our knowledge of the effects of glucose on SS bioavailability. Previously, it was shown in trout that *in vivo* glucose administration elevated plasma SS levels (Harmon *et al.*, 1991). In addition, it has been shown that glucose stimulated the release of SS-14 from the pancreata of fish (Ince and So, 1983; Ronner and Scarpa, 1987; Milgram *et al.*, 1991; Eilertson and Sheridan, 1995) and mammals (Ipp *et al.*, 1977). Glucose also stimulated the release of salmonid SS-25 (a PPSS II" product containing [Tyr^7, Gly^{10}]-SS-14]) from islets isolated from rainbow trout (Eilertson and Sheridan, 1995). Collectively, these findings suggest that glucose regulates the production of SS at multiple levels: at the level of SS gene transcription and at the level of SS secretion.

The regulation of SS biosynthesis and secretion by glucose may have important implications for the nutritional and metabolic physiology of vertebrate organisms. In particular, modulation of SS production by glucose may provide an important feedback control on the release of other metabolically important hormones such as insulin and glucagon in so far as SSs have been shown to inhibit the release of these factors both in mammals (Patel, 1999) and fish (Sheridan *et al.*, 2000). Moreover, the differential effects of glucose on PPSS gene transcription may help to explain both the differential expression of PPSS II mRNAs and the differential responsiveness of SSs (Eilertson and Sheridan, 1993) noted previously.

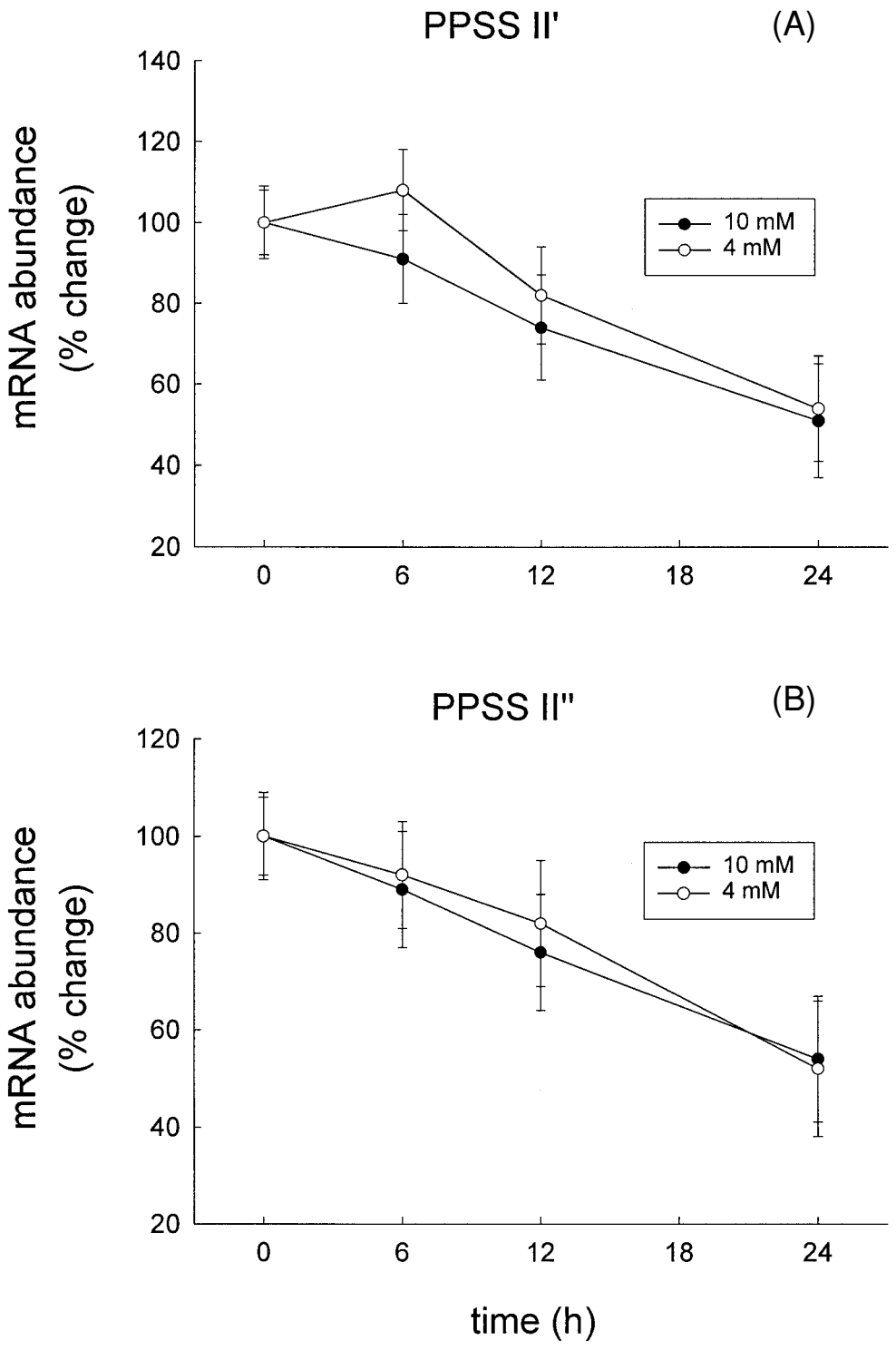

Fig. 2. Glucose does not affect the stability of preprosomatostatin (PPSS) mRNAs. The half-life of (A) PPSS II' and (B) PPSS II" mRNA was determined in rainbow trout islet cells incubated in the presence of 10 mM or 4 mM (control) glucose. Actinomycin D (5 μg/ml), used to inhibit new RNA synthesis, was added to cultures 30 min before time 0; total RNA was extracted at the times indicated and analyzed as described in Materials and Methods. Data are presented as percent change (mean±S.E.M., n=6) from time 0.

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