Glucose regulates pancreatic preprosomatostatin I expression

Gregory T. Melroe, Melissa M. Ehrman[1], Jeffrey D. Kittilson, Mark A. Sheridan*

Department of Zoology and Regulatory Biosciences Center, North Dakota State University, Fargo, ND 58105, USA

Received 26 October 1999; received in revised form 7 December 1999

Edited by Ned Mantei

Abstract Rainbow trout were used as a model system to evaluate the role of glucose in regulating the expression of preprosomatostatin I. Glucose increased pancreatic levels of preprosomatostatin I mRNA in vivo, in concert with elevated plasma somatostatin levels, and in vitro. Glucose-stimulated expression of preprosomatostatin I mRNA required the uptake, phosphorylation, and subsequent metabolism of the sugar in pancreatic islets. These results suggest that glucose modulates both the production and release of somatostatin.

Key words: Somatostatin; mRNA slot-blot quantitation technique; Rainbow trout model system

1. Introduction

The somatostatin (SS) family of peptide hormones has been shown to regulate various aspects of growth, development, and metabolism in vertebrates [1]. Peptides ranging in length from 14 to 37 amino acids and/or varying in amino acid composition, depending on species, have been isolated from numerous tissues, including the central and peripheral nervous systems, the gastrointestinal tract, pancreatic islets, and thyroid tissue [2,3]. The molecular heterogeneity of the SS family stems from tissue-specific variations in biosynthesis from a single larger precursor molecule as well as from the existence of multiple precursors presumably derived from different genes. The different forms of SS observed in mammals (e.g. SS-28) are N-terminal extensions of SS-14, the first somatostatin described, and result from differential processing of the same precursor, preprosomatostatin (PPSS) I [4]. Lampreys, numerous teleost fishes, and frogs possess PPSSs in addition to PPSS I; in the case of teleosts and frogs, the PPSSs derive from different mRNAs [3,5,6].

Relatively little is known about the control of SS biosynthesis and secretion. In mammals, studies have been hampered by the relatively low number of SS-secreting cells in their islets. However, food deprivation as well as in vivo glucose administration were shown to elevate plasma SS-14 levels in mammals [7]. Studies on teleost fishes, which have proved to be particularly good models for the study of SS because they possess a large principal islet (Brockmann body) that is easily separated from exocrine tissue and because this islet possesses a relatively high amount of SS compared to mammalian islets [8], have provided important insight into nutritional regulation of SS secretion. Food deprivation as well as in vivo and in vitro glucose administration have been shown to stimulate the release of SS-14 [9–13]. At present, however, no information exists on the effects of nutrients on SS gene expression or on SS biosynthesis.

In this study, rainbow trout were used as a model system to evaluate the control of SS gene expression. We examined for the first time the effects of glucose on the expression of PPSS I mRNA. The mechanisms by which glucose affects the expression of PPSS I mRNA also were examined. The results of this study contribute to the overall understanding of how the production and secretion of SS is controlled.

2. Materials and methods

2.1. Animals

Juvenile rainbow trout (*Oncorhynchus mykiss)* of both sexes were obtained from Dakota Trout Ranch near Carrington, ND, 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 12:12 h light–dark photoperiod. Fish were fed to satiety twice daily with Supersweet Feeds (Glenco, MN, USA) trout grower, except 24–36 h before in vivo or in vitro experiments. Animals were acclimated to laboratory conditions for at least 4 weeks prior to experiments.

For in vivo experiments, animals were transferred to 50-l aquaria (10 fish/aquarium; one aquarium for each of the following treatments: 3 h saline, 3 h glucose, 12 h saline, and 12 h glucose) and their feeding was suspended. Thirty-six hours later, the fish were anesthetized with 0.05% (v/v) 2-phenoxyethanol (Sigma), weighed, injected (10 μl/g body weight) with either 0.75% (w/v) NaCl (control) or 3000 mg/dl glucose (treated) as described previously [10], and then replaced into their aquarium. At various times after injection, the animals were re-anesthetized and blood and Brockmann bodies were removed. Serum and tissues were immediately frozen on dry ice and stored at −90°C until further analysis, usually within 2 weeks. Hematocrits between the saline- and glucose-injected animals did not differ significantly.

For in vitro experiments, the fish were anesthetized and their Brockmann bodies removed and prepared for culture as described previously [12]. Isolated hemi-islets were placed in 24-well culture plates (ca. 2–3 hemi-islets per well) and preincubated (14°C, 100% O_2, shaken at 120 rpm with a gyratory shaker) for 2 h in 1 ml of basal medium (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, 0.24% bovine serum albumin). Following preincubation, the medium was removed, the islets were washed gently with 1 ml of fresh basal medium, and 1 ml of test medium was added to each well. Test solutions were prepared by adjusting the NaCl concentration so that all solutions were isoosmotic. All of the test agents, including hexoses, analogs and inhibitors, were obtained from Sigma (St. Louis, MO). Incubation proceeded under the same conditions as preincubation for up to 24 h, after which the medium was removed and the islets were immediately frozen on dry ice. Islets were stored at −90°C until RNA extraction and quantitation.

*Corresponding author. Fax: (1)-701-231 7149.
E-mail: msherida@plains.nodak.edu

[1] Present address: School of Medicine, University of Minnesota, Minneapolis, MN 55455, USA.

Abbreviations: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PPSS, preprosomatostatin; SS, somatostatin

PII: S0014-5793(99)01723-8

2.2. mRNA quantitation and plasma hormone and metabolite analyses

Total RNA was extracted from Brockmann bodies using the TRI Reagent® protocol (Molecular Research Center, Inc., Cincinnati, OH). PPSS I mRNA was measured using a quantitative slot-blot technique [6] in which in vitro synthesized cRNA standards were blotted onto a nylon membrane along with sample RNA (Fig. 1B,C). The membranes were first hybridized with a gene-specific ^{32}P-labeled oligonucleotide probe and quantified with the Packard Cyclone Imaging System (Meriden, CT). This probe was highly specific and did not cross-hybridize with other mRNA species (Fig. 1A). Sample blots were then stripped and rehybridized with a full-length ^{32}P-labeled γ-actin probe and requantified. Following correction for background and normalization to γ-actin, mRNA levels were expressed as molecules of mRNA $\times 10^8$ per μg total RNA (Fig. 1).

Plasma concentrations of glucose [14] and SS-14 [12] were measured as previously described.

2.3. Statistics

Statistical differences were estimated by analysis of variance; multiple comparisons among means were made with the Student–Newman–Keuls test. Differences were considered significant at $P < 0.05$. For ease of comparison, data were expressed as % change from control; statistics were performed on untransformed data.

3. Results

3.1. In vivo experiment

The effects of glucose on pancreatic expression of PPSS I mRNA were first studied in vivo. Injection of fish with glucose (3000 mg/dl) resulted in hyperglycemia that lasted for up to 12 h (Fig. 2A), a pattern that was similar to our previous observations [10]. The hyperglycemia was attended by elevated plasma concentrations of SS-14 (Fig. 2B). Alterations in plasma levels of SS-14 corresponded to changes in the steady-state levels of PPSS I mRNA in the Brockmann bodies of trout injected with glucose (Fig. 2C). Whether or not the effects of glucose on the expression of PPSS I mRNA resulted from a direct action was evaluated by in vitro incubation of Brockmann bodies.

3.2. In vitro experiments

Glucose directly stimulated the expression of PPSS I mRNA in Brockmann bodies incubated in vitro. Steady-state levels of PPSS I mRNA increased in a dose-dependent manner (Fig. 3A); the maximum response to glucose was at a concentration of 10 mM. Steady-state levels of PPSS I mRNA rose quickly in response to 10 mM glucose, reaching maximum expression after 6 h; thereafter, RNA levels declined (Fig. 3B). All of the hexoses tested (mannose, galactose, fructose, glucose) induced the expression of PPSS I mRNA; the effects of fructose and galactose were particularly pronounced (Fig. 3C).

The mechanisms by which glucose stimulates the expression of PPSS I mRNA were studied in several experiments. Sucrose, a disaccharide which does not enter cells readily, had no effect on the expression of PPSS I mRNA (Fig. 4A). In addition, neither 3-*o*-methyl glucose, a compound that enters cells but does not get phosphorylated, nor 2-deoxyglucose, a compound which enters cells and gets phosphorylated but not metabolized further, altered the steady-state levels of PPSS I mRNA (Fig. 4A). On the other hand, metabolites such as glycerol, dihydroxyacetone, pyruvate, and lactate, significantly stimulated the levels of PPSS I mRNA (Fig. 4B). An inhibitor of glycolysis, iodoacetate, significantly reduced the levels of

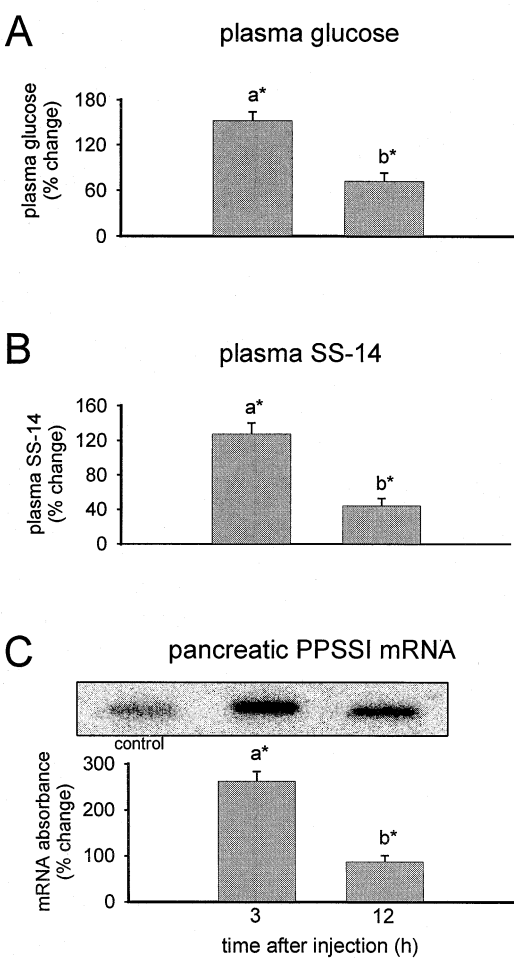

Fig. 2. Effects of glucose injection (3000 mg/dl at 10 μl/g body weight) on the plasma concentration of glucose (A), somatostatin (B), and on the expression of pancreatic PPSS I mRNA (C). Data are presented as % change from saline-injected control (mean ± S.E.M.; $n = 10$) and representative slot blots (inset). Control values at 3 h and 12 h, respectively, for glucose were 111.7 ± 7.2 mg/100 ml and 104.8 ± 6.8 mg/100 ml and for somatostatin were 0.41 ± 0.02 ng/ml and 0.44 ± 0.03 ng/ml. Groups with different letters are significantly ($P < 0.05$) different from one another; *significantly different from control.

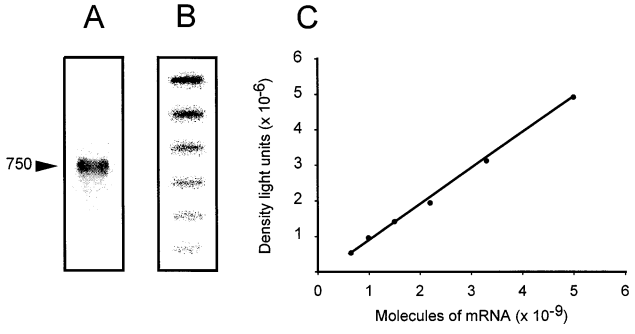

Fig. 1. Quantitative slot-blot analysis of PPSS I mRNA. Serial dilutions of in vitro synthesized cRNA standards (B) and samples were subjected to slot-blot analysis using a ^{32}P-labelled oligonucleotide probe. Northern analysis (A) indicated that this probe was specific for the 745-nt PPSS I transcript. Autoradiograms of blots were quantitated using phosphor imaging and, after correction for background, were plotted (C) as a function of number of molecules/μg total RNA.

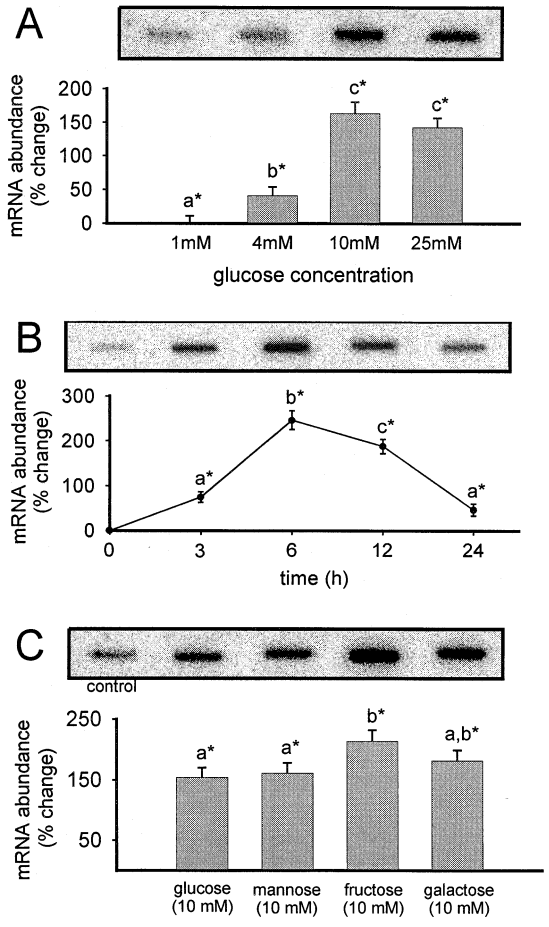

Fig. 3. Effects of glucose (A, B) and of other hexoses (C) on the expression of PPSS I mRNA in rainbow trout Brockmann bodies incubated in vitro. Tissues were incubated for 6 h with the concentration of hexose indicated (A, C) or for various times with 10 mM glucose (B). Data are presented as representative slot blots (insets) and as % change (mean ± S.E.M.; $n = 6$) from 1 mM glucose (A); time 0 h (B); 4 mM glucose control (C). Groups with different letters are significantly ($P < 0.05$) different from one another; *significantly different from control.

PPSS I mRNA (Fig. 4B). Dichloroacetate, which increases flux into the Krebs cycle, as well as intermediates of the Krebs cycle, such as acetate and citrate, also enhanced the levels of PPSS I mRNA (Fig. 4C).

4. Discussion

The results of this study indicate that glucose modulates the expression of the PPSS I mRNA. The effects of glucose are direct and rapid. These findings, the first reporting the effects of metabolites on the levels of PPSS mRNAs, suggest that glucose stimulates the biosynthesis as well as the secretion of somatostatins, peptides important in the regulation of growth, development, and metabolism of vertebrates [1].

Glucose-stimulated expression of PPSS I mRNA requires cellular entry and the subsequent metabolism of the sugar. This conclusion is supported by several observations. First, sucrose, a disaccharide which does not enter cells readily, had no effect on PPSS I expression; this lack of an effect also excludes the possibility of an osmotic action. Second, a variety of hexoses, including glucose, galactose, and fructose, which enter cells and undergo phosphorylation, isomerization and metabolism to varying degrees, all stimulated PPSS I mRNA expression. Third, that hexose-stimulated PPSS I mRNA expression requires phosphorylation was supported by the failure of the glucose analog, 3-*o*-methyl glucose, to alter steady-state levels of PPSS I mRNA. Phosphorylation alone, however, does not appear to be sufficient for hexose-stimulated PPSS I mRNA expression. This is indicated by the inability of the glucose analog, 2-deoxyglucose, to affect PPSS I mRNA expression. Lastly, the requirement for the subsequent metabolism of glucose through glycolysis and Krebs cycle was indicated by the ability of metabolites such as dihydroxyacetone, glycerol, lactate, pyruvate, acetate, and citrate to stimulate the levels of PPSS I mRNA. Inhibition of glycolysis with iodoacetate significantly reduced the levels of PPSS I mRNA. Moreover, increased flux through the Krebs cycle, via dichloroacetate action on mitochondrial pyruvate dehydrogenase activity, stimulated the levels of PPSS I mRNA. Together, the present findings suggest that the proximate mediator of glucose-stimulated PPSS I expression is generated after the aldolase step of glycolysis.

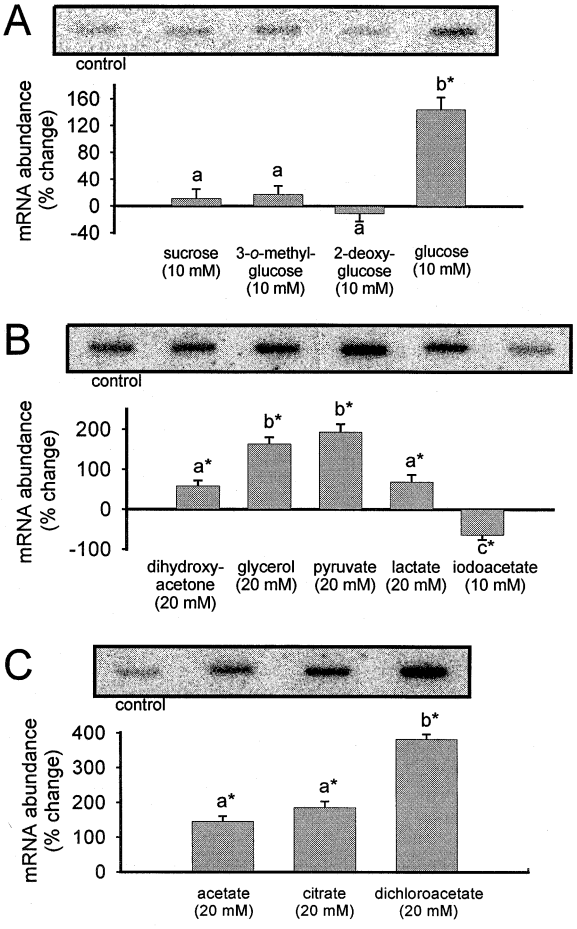

Fig. 4. Effects of sucrose and various glucose analogs (A) and of selected intermediates, inhibitors, or stimulators of glycolysis (B) and the Krebs cycle (C) on the expression of PPSS I mRNA in rainbow trout Brockmann bodies incubated in vitro for 6 h. Data are presented as representative slot blots (insets) and as % change (mean ± S.E.M.; $n = 6$) from control (4 mM glucose). Groups with different letters are significantly ($P < 0.05$) different from one another for a given mRNA species; *significantly different from control.

The exact mechanism(s) by which glucose stimulates steady state levels of PPSS I mRNA is (are) not known. Whether the proximate mediators produced during the metabolism of glucose activate specific *trans*-acting factors that, in turn, modulate gene expression via carbohydrate-responsive elements in promoter regions of the SS gene is a possibility that requires further study. It also should be noted that an influence of glucose metabolism on the stability of PPSS I mRNA cannot be ruled out.

The finding that glucose stimulates the expression of PPSS I mRNA extends our knowledge of the effects of glucose on SS production. Previously, glucose administration in vivo has been shown to elevate plasma somatostatin levels in fish [10] and mammals [7]. In addition, glucose stimulated the secretion of SS-14, a principal SS form located at the C-terminus of PPSS I, directly from pancreatic preparations of numerous species of fish [9,11,12,15] and mammals [16]. The present findings indicate that glucose-stimulated release of SS-14, both in vivo and in vitro, results, at least in part, from the increased expression of PPSS I mRNA. The regulation of SS biosynthesis and secretion by glucose has important implications for the nutritional and metabolic physiology of vertebrate organisms. Glucose-modulated SS production provides 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 fish [17] and in mammals [2].

Acknowledgements: We thank Leslie Alexander, Kristen Baltrusch, and Anne Littlefield for their technical assistance and Dr. Gerald Taborsky, Jr. (Veterans Affairs Medical Center, Seattle, WA) for his generous donation of SS-14 antiserum. This research was supported by grants from the U.S. National Science Foundation (IBN 9723058) and the U.S. Department of Agriculture (98-03652) to M.A.S.

References

[1] Sheridan, M.A., Kittilson, J.D. and Slagter, B.A. (1999) Am. Zool. (in press).
[2] Patel, Y.C. (1992) in: Basic and Clinical Aspects of Neuroscience (Weil, C., Muller, E.E. and Thorner, M.O., Eds.), Vol. 4, pp. 1–16, Springer-Verlag, Berlin.
[3] Conlon, J.M., Tostivint, H. and Vaudry, H. (1997) Regul. Pept. 69, 95–103.
[4] Conlon, J.M. (1989) in: Comparative Physiology of Regulatory Peptides (Holmgren, S., Ed.), pp. 174–202, Chapman and Hall, New York.
[5] Kittilson, J.D., Moore, C.A. and Sheridan, M.A. (1999) Gen. Comp. Endocrinol. 114, 88–96.
[6] Moore, C.A., Kittilson, J.D., Ehrman, M.M. and Sheridan, M.A. (1999) Am. J. Physiol. (in press).
[7] Gerich, J.H. (1983) in: Diabetes Mellitus: Theory and Practice, 3rd edn. (Ellenberg, M. and Rifken, H., Eds.), pp. 225–254, Medical Examinations, New York.
[8] Plisetskaya, E.M., Pollock, H.G., Rouse, J.B., Hamilton, J.W., Kimmel, J.R., Andrews, P.C. and Gorbman, A. (1986) Gen. Comp. Endocrinol. 63, 242–263.
[9] Ronner, P. and Scarpa, A. (1987) Gen. Comp. Endocrinol. 65, 354–362.
[10] Harmon, J.S., Eilertson, C.D., Sheridan, M.A. and Plisetskaya, E.M. (1991) Am. J. Physiol. 261, R609–R613.
[11] Milgram, S.L., McDonald, J.K. and Noe, B.D. (1991) Am. J. Physiol. 261, E444–E456.
[12] Eilertson, C.D. and Sheridan, M.A. (1995) Am. J. Physiol. 269, R1017–R1023.
[13] Pesek, M.J. and Sheridan, M.A. (1996) J. Endocrinol. 150, 179–186.
[14] Hüvarinen, A. and Nikkila, E.A. (1962) Clin. Chim. Acta 7, 140–143.
[15] Ince, B.W. and So, S.T. (1983) Gen. Comp. Endocrinol. 53, 389–397.
[16] Ipp, E., Dobbs, R.E., Arimura, A., Vale, W., Harris, V. and Unger, R.A. (1977) J. Clin. Invest. 60, 760–765.
[17] Eilertson, C.D. and Sheridan, M.A. (1993) Gen. Comp. Endocrinol. 92, 62–70.