Volume 292, number 1,2, 264–266 FEBS 10383 November 1991

ADONIS 001457939101091V

Increase of β-actin mRNA upon hypotonic perfusion of perfused rat liver

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Received 12 September 1991

β-Actin mRNA levels in livers exposed to hypotonic perfusion (from 305 to 225 mosmol/l) for one hour are increased 2-fold relative to albumin mRNA. Like albumin, glyceraldehyde-3-phosphate dehydrogenase and tyrosine aminotransferase mRNAs remain at the levels observed under normotonic conditions. The increase in β-actin mRNA is interpreted as a cytoskeletal response due to cell swelling.

β-Actin; Hypotonic; Liver perfusion; Albumin; Tyrosine aminotransferase; Glycerinaldehyde-3-phosphate dehydrogenase

1. INTRODUCTION

Like most mammalian cells, hepatocytes are able to regulate their volume within minutes when exposed to anisotonic media [1]. These volume-regulatory responses, however, only partially restore cell volume, i.e. the cells remain in a slightly swollen state throughout hypotonic exposure. Recent studies have shown that cell swelling, as it occurs not only during hypotonic exposure but also during cumulative substrate uptake or under the influence of insulin, modulates metabolic liver cell function (for reviews, see [2,3]). Here, we report an increase in β-actin mRNA levels in response to hypotonic cell swelling in perfused rat liver.

2. MATERIALS AND METHODS

2.1. *Liver perfusion*

Livers from male Wistar rats, 150–250 g body weight, fed ad libitum, were perfused at 37°C in a non-recirculating system from the portal to the hepatic vein with hydrogen-carbonate-buffered Krebs-Henseleit saline containing L-lactate and pyruvate [4]. The influent perfusate was equilibrated with O_2/CO_2 (95/5 v/v). Hypotonic perfusion was performed by decreasing the NaCl concentration of the influent perfusion medium by 40 mmol/l, resulting in a decrease of perfusate osmolarity by 80 mosmol/l.

2.2. *RNA analysis*

Total RNA was isolated from rat liver as described [5], and was either separated on 1% agarose/formaldehyde gels and transferred to Genescreen-plus membranes for Northern hybridization or denaturated by formamide/formaldehyde and applied directly to the membrane for dot blot hybridization. Membranes were hybridized under stringent conditions as described [6] using random oligonucleotide-primed ^{32}P-labeled insert probes. Washed filters were exposed to Kodak XR film and hybridization was quantified by laser densitometry within the linear range.

The probes used were pβ-MA containing a 420 bp insert of mouse β-actin cDNA [7], BlualbHind containing a 300 bp insert from mouse albumin cDNA [8], pRLCGAP containing the 1.3 kb rat glyceraldehyde-3-phosphate dehydrogenase cDNA [9], kindly provided by Dr. R. Wu (Ithaca, NY), and pTATc3 containing a 600 bp insert from rat tyrosine aminotransferase cDNA [10], kindly provided by Dr. G. Scherer (Freiburg, Germany).

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3. RESULTS

In RNA isolated after 1 h of perfusion the amount of β-actin mRNA (Fig. 1A) in relation to that of albumin (Fig. 1B) or glyceraldehydephosphate dehydrogenase (Fig. 1C) was increased approximately 2-fold in hypotonically as compared to normotonically perfused livers. The ratios of glyceraldehydephosphate dehydrogenase mRNA to the mRNAs for albumin and tyrosine aminotransferase were unaltered (Fig. 1, Table I). This indicates that the difference between glyceraldehydephosphate dehydrogenase and β-actin message is due to an increase in β-actin mRNA and not due to a decrease in glyceraldehydephosphate dehydrogenase mRNA. The level of glyceraldehydephosphate dehydrogenase mRNA closely paralleled the amounts of total RNA as revealed by visual inspection of ethidium bromide stained gels.

The time course of the increase in β-actin message is shown in Fig. 2. Livers were perfused for 15, 30, 60 and 120 min, and total RNA preparations were examined for β-actin and albumin mRNA by dot blot hybridization. The relative amount of β-actin mRNA had already increased after 15 min and continued to increase throughout the experiment. Normotonic perfusion showed an approximately stable ratio; a slight increase of the β-actin/albumin mRNA ratio during the first hour of normotonic perfusion (only 2 livers) was not further studied.

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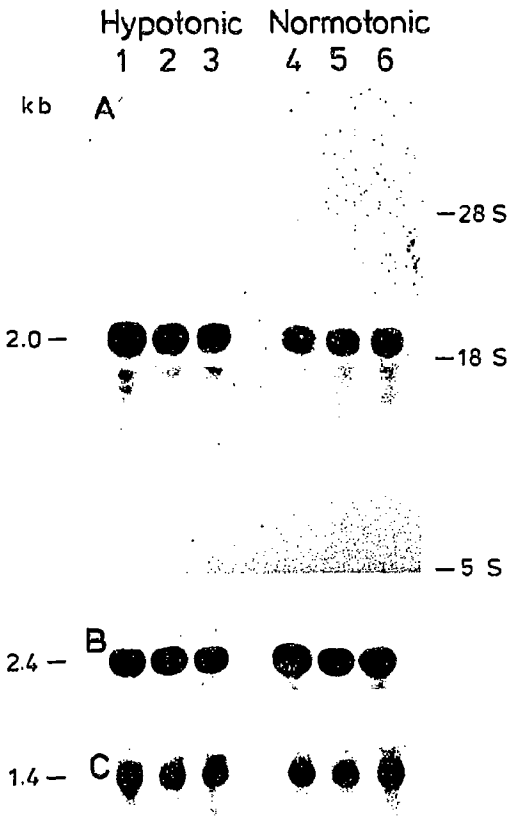

Fig. 1. Northern blot analysis of mRNA changes in hypotonic perfusion. Three rat livers each were perfused for 1 h with hypotonic (1–3) or normotonic (4–6) medium, respectively. Hybridization was performed with probes for (A) β-actin; (B) albumin; and (C) glyceraldehydephosphate dehydrogenase.

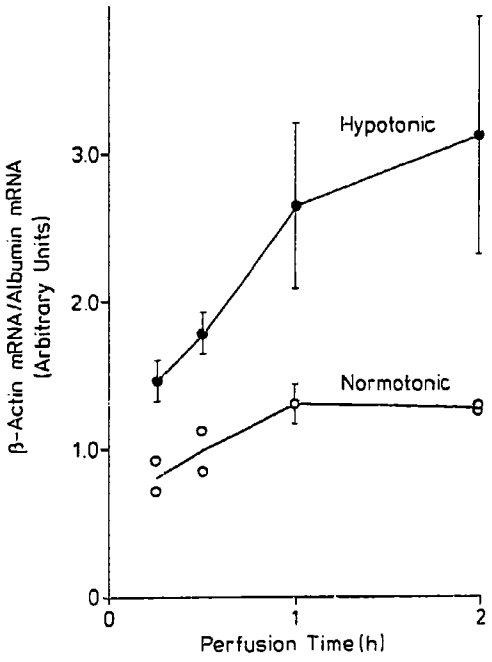

Fig. 2. Time course of β-actin mRNA level in relation to albumin mRNA level in perfused rat livers. Perfusions in hypotonic (●) or normotonic (○) conditions. Data as obtained from dot blots by laser densitometric analysis. Results are presented as means ± SE (n=3–4) or as two individual points, from different livers.

4. DISCUSSION

As shown recently [1], hypotonic exposure leads to a volume-regulatory K^+ efflux from the liver of 13.3±0.7 μmol/g liver, which is completed during the first 10 min of hypotonic exposure. Thereafter, liver cell volume has reached a new steady state, which is 16.5±2.6% above the volume during normotonic perfusion [11], so that cells are exposed to mechanical stress. The elevated level of β-actin mRNA is significant at 1 h and is interpreted as a cellular response to the persistent mechanical stress resulting from cell swelling. β-Actin mRNA has been reported to be induced during cell damage by various agents [12,13] as well as during establishment of hepatocytes in primary cultures [14]. Interestingly, in the latter case the effect was about 3-fold, similar to that in hypotonically perfused livers (Table I, Figs. 1,2), and was attributed to the change in cell shape and related general cytoskeletal rearrangements. It is therefore possible that similar changes in the expression of cytoskeletal proteins are elicited by osmotically induced cell volume changes as studied here. Recent work using the rat pheochromocytoma cell line PC 12A indicated reorganization of microfilaments, but not of microtubules during hypo-osmotic cell swelling [15]. A role of microfilaments in the process of cell volume regulation is also suggested by the finding that cytochalasin B inhibits the regulatory volume decrease in Necturus gall bladder epithelial cells [16].

An increased level of β-actin mRNA may be the result of either an increased transcription rate, a decrease in the degradation of the mRNA, or a combination of both. Albumin mRNA is the most abundant mRNA in hepatocytes, with a slow turnover [17], whereas tyrosine aminotransferase mRNA is moderately abundant with a rather fast turnover of 1 h [18]. Therefore, a general

Table I

Northern blotting analysis of total RNA from perfused rat livers

	Density (arbitrary units)	
	Normotonic	Hypotonic
β-Actin	0.9 ± 0.1	2.2 ± 0.5
Albumin	0.5 ± 0.1	0.5 ± 0.1
Glyceraldehydephosphate dehydrogenase	≡ 1.0	≡ 1.0
Tyrosine aminotransferase	0.9 ± 0.2	1.0 ± 0.2

Values at 1 h of perfusion are the results of densitometric analysis of autoradiographs normalized to glyceraldehydephosphate dehydrogenase mRNA, arbitrarily set equal to 1.0 for both normotonically and hypotonically perfused livers.

inhibition of mRNA turnover is probably not involved in the increase in β-actin mRNA level upon cell swelling.

Acknowledgement: Support by the Jung-Stiftung für Medizinische Forschung, Hamburg, and by the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

REFERENCES

[1] Lang, F., Stehle, T. and Häussinger, D. (1989) Pflügers Arch. 413, 209–216.
[2] Häussinger, D. and Lang, F. (1991) Biochem. Cell. Biol. 69, 1–4.
[3] Häussinger, D. and Lang, F. (1991) Cell. Physiol. Biochem. 1, 121–130.
[4] Sies, H. (1978) Methods Enzymol. 52, 48–59.
[5] Weidner, U., Peter, S., Strohmeyer, T., Hussnätter, R., Ackermann, R. and Sies, H. (1990) Cancer Research 50, 4504–4509.
[6] Schulz, W.A. and Gais, G. (1989) Biochim. Biophys. Acta 1013, 125–132.
[7] Sakiyama, S., Nakumura, Y., Tokunaga, K., Takazawa, H., Ohwaki, Y. and Nagano, T. (1989) Cell Tissue Res. 258, 225–231.
[8] Schmid, P. and Schulz, W.A. (1990) Differentiation 45, 96–102.
[9] Tso, J.Y., Sun, X.H., Kao, T.H., Reece, K.S. and Wu, R. (1985) Nucleic Acids Res. 13, 2485–2502.
[10] Scherer, C., Schmid, W., Strange, C.M., Röwekamp, W. and Schütz, G. (1982) Proc. Natl. Acad. Sci. USA 79, 7205–7208.
[11] vom Dahl S., Hallbrucker, C., Lang, F., Gerok, W. and Häussinger, D. (1991) Biochem. J. 278, 771–777.
[12] Mai, S., Lücke-Huhle, C., Kaina, B., Rahmsdorf, H.J., Stein, B., Ponta, H. and Herrlich, P. (1990) Ionizing radiation damage to DNA: Molecular Aspects, pp. 319–331, Wiley-Liss, New York.
[13] Fornace, A.J., Nebert, D.W., Hollander, M.C., Luethy, J.D., Papathanasiou, M., Fargnoli, J. and Holbrook, N.J. (1989) Mol. Cell. Biol. 9, 4196–4203.
[14] Fraslin, J.-M., Kneip, B., Vaulont, S., Glaise, D., Munnich, A. and Guguen-Guillouzo, C. (1985), EMBO J. 4, 2487–2491.
[15] Cornet, M., Delpire, E. and Gilles, R. (1987) Pflügers Arch. 410, 223–225.
[16] Foskett, J.K. and Spring, K.R. (1985) Am. J. Physiol. 248, C27-C36.
[17] Powell, D.J., Friedman, J.M., Oulette, A.J., Krauter, K.S. and Darnell Jr., J.E. (1984) J. Mol. Biol. 179, 21–35.
[18] Lee, K.-L., Isham, K. R., Johnson, A. and Kenney, F.T. (1986) Arch. Biochem. Biophys. 248, 597–603.