Hypoosmolarity and glutamine increased the β-actin gene transcription in isolated rat hepatocytes

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Abstract The mechanism of action of hydration state was studied on β-actin gene expression in isolated hepatocytes. Results obtained with Northern blot analysis and run on transcription assays show that hypoosmolarity increased and hyperosmolarity decreased the β-actin mRNA level through a corresponding modulation of the rate of the gene transcription. Glutamine, which is known to induce cell swelling, also increased the β-actin mRNA level in a dose-dependent manner and induced a stimulation of the β-actin gene transcription. Thus, cell hydration state regulates gene expression in the liver through a transcriptional mechanism.

Key words: Hydration state; Glutamine; Hepatocyte; Transcription; β-Actin

1. Introduction

Hydration state has been shown to regulate gene expression in the liver. Thus, hypoosmolarity has been reported to increase the actin mRNA level in isolated rat hepatocytes [1], the argininosuccinate synthetase mRNA level in cultured rat hepatocytes [2] and the c-*jun* mRNA level in rat hepatoma cells [3]. Conversely, hypoosmolarity has been reported to decrease the phosphoenolpyruvate carboxykinase [4,5] and the tyrosine aminotransferase mRNA levels [5] in both perfused rat liver and H4IIE rat hepatoma cells. The opposite was observed in hyperosmotic conditions. These results suggested that hydration state of the liver may potentially regulate gene transcription and (or) mRNA stabilization.

The aim of this work was to demonstrate that the transcription of the β-actin gene was regulated by the hydration state of the hepatocytes. The obtained results in isolated rat hepatocytes demonstrated that hypoosmolarity increased and hyperosmolarity decreased β-actin gene transcription. These results also show that glutamine, a compound known to induce cell swelling, increased the rate of actin gene transcription.

2. Materials and methods

2.1. Materials

α-Amanitin, cordycepin and glutamine were purchased from Sigma and actinomycin D from Calbiochem. Guanidinium thiocyanate was from Fluka (Basel, Switzerland). Hybond-N membranes, multiprime DNA-labelling system, [α-^{32}P]dCTP (3000 Ci/mmol), [α-^{32}P]UTP (800 Ci/mmol) and Hyperfilm MP were from Amersham. The probes used were the full-length chicken β-actin cDNA provided by Dr. D.W. Cleveland [6], an insert of rat glyceraldehyde-3-phosphate dehydrogenase (GraP-DH) (EC 1.2.1.12) provided by Dr. J.M. Blanchard [7] and an insert of 18S rRNA provided by Dr. L. Hendriks [8], both used as internal control.

2.2. Isolated liver cells

Hepatocytes were prepared as described previously [9] from 24 h starved male Wistar rats (200–220 g). The cells (usually 50–70 mg wet weight/ml) were shaken (165 strokes/min) in stoppered scintillation vials at 37°C for the indicated times. The standard incubation medium was a Krebs-Henseleit bicarbonate buffer at pH 7.4, containing 5 mM glucose. Hypo- and hyperosmotic media were obtained by decreasing (−50 mM) or increasing (+35 mM) the NaCl concentration of the buffer, respectively. All media were in equilibrium with a gas phase of O_2/CO_2 (19:1). For RNA determination and nuclear run on transcription assay, the cells were spun down rapidly at 4°C (2000×*g*, 30 s) at the end of the incubation period and the pellets stored at −80°C.

2.3. Extraction and analysis of cellular RNA

Isolation of total RNA was performed by a guanidinium thiocyanate procedure [10]. RNA was separated on 1.5% agarose/formaldehyde gels and transferred to nylon membrane for Northern hybridization. Membranes were hybridized using random oligonucleotide-primed ^{32}P-labelled insert as described [11]. Filters were washed and exposed to Hyperfilm at −80°C using intensifying screens. Relative densities of the hybridization signals were quantified by scanning the films with a Shimadzu densitometer. To correct for differences in RNA loading, all the results were expressed as the ratio of the scanned values for β-actin mRNA versus those for GraP-DH mRNA (relative level).

2.4. Nuclear run on transcription assay

The preparation of nuclei as well as RNA polymerase elongation reaction were performed essentially as described by Vannice et al. [12]. For transcription, 2×10^7 nuclei were incubated for 30 min at 28°C in a 200 μl reaction mixture containing 15% glycerol, 50 mM HEPES pH 8.0, 150 mM KCl, 1 mM dithiothreitol, 2.5 mM Mg-acetate, 1 mM $MnCl_2$, 0.5 mM EDTA, 4 mM creatine phosphate, 15 U/ml creatine kinase, 1 mM spermidine, 0.5 mM each of ATP, CTP and GTP, and 500 U/ml RNasin in the presence of 100 μCi of [α-^{32}P]UTP. Then, 40 U DNAse I were added and the samples were incubated at 37°C for 15 min. The labelled RNAs were extracted essentially as described by Chomczynsky and Sacchi [10]. 5 μg of denatured plasmids was spotted onto cellulose using a slot blot apparatus. Each DNA bearing filters were prehybridized then hybridized with 5×10^6 cpm of labelled RNA at 42°C for 3 days. Hybridization was carried out with three separate plasmids including the pGEM vector with the β-actin cDNA, a pBR322 as control for non-specific binding of labelled RNA and the 18S rRNA probe as internal control. Filters were then washed and exposed to Hyperfilm at −80°C with intensifying screens. The relative amount of labelled nuclear transcripts hybridized to plasmids bound to nitrocellulose was determined by densitometric scanning of autoradiograms. The obtained values for the β-actin signal were corrected using the 18S values.

2.5. Expression of the results

The results are expressed as means ± S.E.M. for the observations on the indicated number (*n*) of different cell preparations. Statistical significance of differences was calculated by Student's *t*-test for paired data.

3. Results and discussion

3.1. Actinomycin D totally blocked the increase in the β-actin mRNA level induced by hypoosmolarity

Hepatocytes were incubated for 120 min in the presence of

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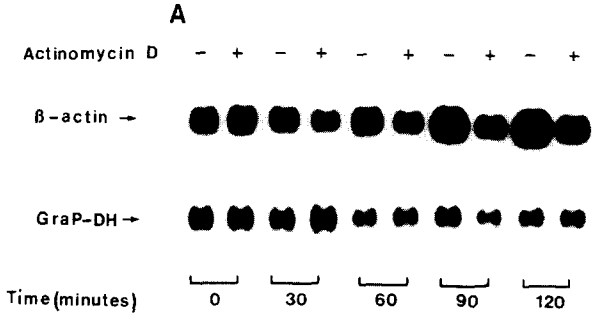
5 mM glucose in hypo- (−50 mM NaCl) or hyperosmotic media (+35 mM NaCl). In hypoosmotic medium, the β-actin mRNA level was two-fold higher than that measured in hyperosmotic medium (Table 1). These results confirmed those reported by Theodoropoulos et al. [1]. Table 1 also shows that the increase in the β-actin mRNA level observed in hypoosmotic medium disappeared in the presence of actinomycin D, a known inhibitor of gene transcription: in this experimental condition, the β-actin mRNA level was not significantly different from that observed in hyperosmotic condition. The same inhibitory effect was observed in the presence of cordycepin and α-amanitin, two other known inhibitors of transcription (not shown). Time course study revealed that hypoosmolarity significantly increased the β-actin mRNA level in the first minutes of the incubation period (Fig. 1). Moreover, this figure also shows that actinomycin D totally blocked the stimulatory effect of hypoosmolarity in the first minutes of the incubation period. Taken together, these results strongly suggested that hypoosmolarity increased the β-actin mRNA level through an increase in its gene transcription. The fact that actinomycin D did not modify the β-actin mRNA level in hyperosmotic conditions (Table 1) also suggested that hyperosmolarity might, in opposite, decrease the rate of the β-actin gene transcription.



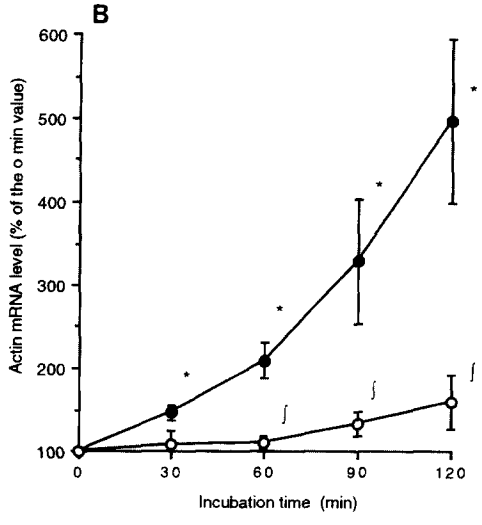


Fig. 1. Time course study of the effect of actinomycin D on the β-actin mRNA level in hepatocytes incubated in hypotonic conditions. Hepatocytes were incubated for different periods of time in a hypoosmotic medium, without (●) or with (○) 0.5 μg/ml actinomycin D. Total RNA was extracted and 20 μg aliquots analyzed by Northern blot. They were probed successively with the β-actin and the GraP-DH cDNAs. A: Representative autoradiogram. B: Scanned data of autoradiograms. Values are expressed as percent of the measured values at the initial period of time. The results are means ± S.E.M. for 6 different cell preparations. *Significantly different ($P < 0.05$) from the value measured at 0 min; ^{f}significantly different ($P < 0.05$) from the corresponding control value.

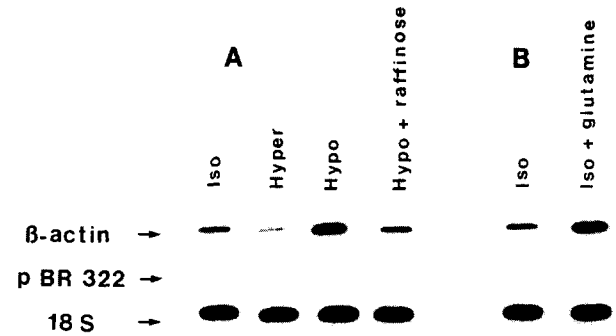


Fig. 2. Influence of anisotonicity and glutamine on the rate of β-actin gene transcription. Hepatocytes were incubated for 90 min, either in different osmotic conditions (Hypo, Hyper, A), or in isosmotic medium (iso) without or with 10 mM glutamine (B). 80 mM raffinose was added to hypoosmotic medium to restore the isosmotic condition. After extraction, nuclei were incubated with [α-^{32}P]UTP, as described. The labelled RNAs were hybridized on to filters containing the β-actin cDNA, pBR322 for non-specific binding and the 18S rRNA probe as internal control.

3.2. *Hydration state of hepatocytes regulated β-actin gene transcription*

In order to specify whether a transcriptional mechanism was involved in the effect of hypoosmolarity, run on transcription assays were performed. Hepatocytes were incubated for 90 min in hypo- or hyperosmotic media. As shown in Fig. 2A for a representative experiment, the rate of transcription was 4.6-fold higher in hepatocytes incubated in hypoosmotic conditions than that measured in hyperosmotic conditions (1, hyper; 4.62 ± 1.07, hypo; $n = 5$; $P < 0.05$). This demonstrated that hypoosmolarity increased the β-actin mRNA level through a transcriptional mechanism. Fig. 2A also shows

Table 1
Influence of actinomycin D on the β-actin mRNA level in isolated rat hepatocytes incubated in hypo- and hyperosmotic conditions

Condition	Actinomycin D	
	−	+
Hypoosmotic	3.47 ± 0.50	1.56 ± 0.30*
Hyperosmotic	1.75 ± 0.26^{f}	1.72 ± 0.29

Hepatocytes were incubated at 37°C for 120 min in the presence of 5 mM glucose in hypo- (−50 mM NaCl) and hyperosmotic (+35 mM NaCl) media, without or with 0.5 μg/ml actinomycin D. Total RNAs were extracted and analyzed by Northern blot. They were probed successively with the β-actin and the GraP-DH cDNAs. Results are given in arbitrary units which represent the means ± S.E.M. for six different cell preparations.
*Significantly different ($P < 0.05$) from the values obtained with cells without actinomycin D. ^{f}Significantly different ($P < 0.05$) from the hypoosmotic condition.

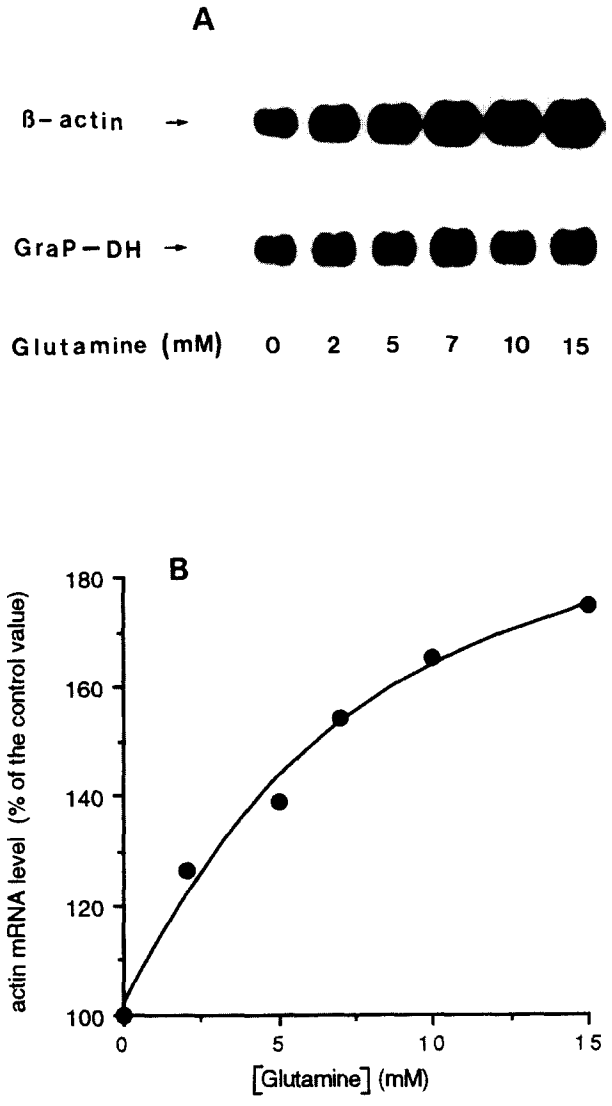


Fig. 3. Dose response of the effect of glutamine on the β-actin mRNA level. Hepatocytes were incubated for 120 min in the presence of different glutamine concentrations and total RNA was extracted for Northern blot analysis. A: Representative autoradiogram. 20 μg total RNA was applied to each lane. They were probed successively with the β-actin and the GraP-DH cDNAs. B: Scanned data of autoradiograms; results are expressed as percent of control value and represent the means of two different cell preparations.

that, in fact, hypoosmolarity increased and hyperosmolarity decreased the rate of the β-actin gene transcription as compared with that measured in isosmotic conditions (1, iso; 2.27 ± 0.24, hypo; 0.43 ± 0.07, hyper; $n = 4$; $P < 0.05$). These results suggested that hydration state of the cells regulates β-actin gene transcription since hypoosmolarity induces cell swelling and hyperosmolarity cell shrinkage. However, the hypo- and hyperosmotic media were obtained by decreasing and increasing the NaCl concentration, respectively. Thus, an effect of the change in the NaCl concentration could not be excluded to explain the observed modulation of the β-actin gene transcription. The effect of raffinose addition was therefore tested on the β-actin gene transcription in hypoosmotic conditions. As shown in Fig. 2A, addition of 80 mM raffinose to hypoosmotic medium inhibited the stimulatory effect of hypoosmolarity on the rate of the β-actin gene transcription (1, iso; 2.62, hypo; 1.24, hypo+raffinose; $n = 2$). This demonstrated that hydration state of the hepatocytes regulates the β-actin gene transcription.

3.3. *Glutamine increased the β-actin mRNA level through a transcriptional mechanism*

Glutamine, which is known to induce cell swelling, has been reported to increase the β-actin mRNA level in isolated rat hepatocytes [1]. Since the experiments described above demonstrated that cell swelling increased the β-actin gene transcription, nuclear run on assay was used in order to demonstrate that this mechanism was also involved in the effect of glutamine. As shown in Fig. 2B for a representative experiment, 10 mM glutamine increased the rate of the β-actin gene transcription (1, iso; 2.26, glutamine; $n = 2$). Moreover, this observed increase in the β-actin gene transcription was associated with an increase in the corresponding mRNA level (1.60 ± 0.09, control; 3.17 ± 0.048, glutamine; $n = 6$; $P < 0.05$), as shown by Northern analysis. Fig. 3 specifies that this effect of glutamine on the β-actin mRNA level was dose-dependent and its half-maximum stimulatory effect was obtained at about 3 mM. Interestingly, this value was reported for the half-maximum effect of glutamine on the argininosuccinate synthetase mRNA level in cultured rat hepatocytes; in these experiments, the glutamine-induced cell swelling had been reported to be responsible for this effect [2].

In conclusion, the data reported here demonstrated that hypoosmolarity increased and hyperosmolarity decreased the β-actin mRNA level through a corresponding modulation of gene transcription. This is, to our knowledge, the first report that hydration state regulates gene expression in the liver through a transcriptional mechanism. Moreover, this work also demonstrated that this mechanism might be of physiological significance: indeed, glutamine, the most abundant circulating amino acid, might increase the β-actin gene transcription through this mechanism.

References

[1] Theodoropoulos, P.A., Stournaras, C., Stoll, B., Markogiannakis, E., Lang, F., Gravanis, A. and Häussinger, D. (1992) FEBS Lett. 311, 241–245.
[2] Quillard, M., Husson, A. and Lavoinne, A. (1996) Eur. J. Biochem. 236, 56–59.
[3] Finkenzeller, G., Newsome, W., Lang, F. and Häussinger, D. (1994) FEBS Lett. 340, 163–166.
[4] Newsome, W.P., Warskulat, U., Noe, B., Wettstein, M., Stoll, B., Gerok, W. and Häussinger, D. (1994) Biochem. J. 304, 555–560.
[5] Warskulat, U., Newsome, W., Noe, B., Stoll, B. and Häussinger, D. (1996) Biol. Chem. Hoppe-Seyler 377, 57–65.
[6] Cleveland, D.W., Lopata, M.A., MacDonald, R.J., Cowan, N.J., Rutter, W.J. and Kirschner, M.W. (1980) Cell 20, 95–105.
[7] Fort, Ph., Marty, L., Piechaczyk, M., El Sabrouty, S., Dani, Ch., Jeanteur, Ph. and Blanchard, J.M. (1985) Nucleic Acids Res. 13, 1431–1442.
[8] Hendriks, L., Van de Peer, Y., Van Herck, M., Neefs, J.M. and De Wachter, R. (1990) FEBS Lett. 269, 445–449.
[9] Marchand, J.C., Lavoinne, A., Giroz, M. and Matray, F. (1979) Biochimie 61, 1273–1282.
[10] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[11] Renouf, S., Buquet, C., Fairand, A., Benamar, M. and Husson, A. (1993) Biochem. J. 291, 609–613.
[12] Vannice, J.L., Taylor, J.M. and Ringold, G.M. (1984) Proc. Natl. Acad. Sci. USA 81, 4241–4245.