Na^+,K^+-ATPase expression during the early phase of liver growth after partial hepatectomy

Joan Vicenç Martínez-Mas, Julia Peinado-Onsurbe, Bonaventura Ruiz-Montasell, Antonio Felipe, F. Javier Casado, Marçal Pastor-Anglada*

Unitat de Bioquímica i Biologia Molecular, Departament de Bioquímica i Fisiologia, Universitat de Barcelona, Diagonal 645, 08071 Barcelona, Spain

Received 3 January 1995; revised 21 February 1995

Abstract **Na^+,K^+-ATPase expression has been studied in the early phase of liver growth after partial hepatectomy to ascertain whether its increased activity is due to stable effects, involving de novo synthesis and insertion of pumps into the plasma membrane. Na^+,K^+-ATPase activity progressively increases after partial hepatectomy, reaching a three-fold induction above basal values 12 h after surgery. mRNA amounts of both α_1 and β_1 subunits are rapidly increased up to two-fold for α_1 and nearly three-fold for β_1, at 9 and 12 h post-hepatectomy, respectively. This correlates with increased abundance of both subunit proteins. The results prove that the increase of Na^+,K^+-ATPase activity correlates with higher expression of both subunit proteins and mRNAs, although the characteristics of the induction suggest that some translational and post-translational events may be equally involved in the increased activity of the pump.**

Key words: Na^+,K^+-ATPase; Liver regeneration; Gene expression

1. Introduction

Transient activation of Na^+ influx, Na^+, K^+-ATPase and selective induction of potential-sensitive plasma membrane transporters is an early feature of liver regeneration after partial hepatectomy [1,2]. These adaptations have been considered as permissive steps for liver growth and may constitute key events in the control of normal hepatocyte proliferation. Nevertheless, the hepatocyte undergoes more permanent changes at the plasma membrane able to sustain enhanced solute uptake during the pre-replicative phase of liver regeneration. Induction of system A for neutral amino acid transport is stable in plasma membrane vesicles [3,4] and independent of the Na^+-transmembrane gradient [4]. Similarly, the concentrative Na^+-dependent nucleoside transport system of liver parenchymal cells shows a stable induction early after partial hepatectomy [5]. Both transport systems are responsible for the active uptake of substrates relevant to cell proliferation, i.e. amino acids and nucleosides. Changes in the protein profile and enzyme activities of the sinusoidal plasma membrane of the hepatocyte have been related to the remodeling of the cell surface prior to mitosis [6], but they may also obey to metabolic purposes.

Hepatocyte Na^+,K^+-ATPase activity is increased after partial hepatectomy [2,6,7], probably as a way to compensate for the metabolic pressure induced by the stable increase of several Na^+-coupled solute transporters [4,5]. The activity of the pump is under complex control, involving transcriptional, translational and post-translational steps. At this stage it is not clear whether the increased Na^+, K^+-ATPase activity occurring in early phases of liver cell proliferation correlates with enhanced expression of their subunits. The aim of this study was to address this issue by monitoring the changes in the amount of mRNA and protein for both subunits, alpha and beta, during the pre-replicative phase of liver growth.

2. Materials and methods

2.1. Animals and surgery

Overnight fasted male Wistar rats (200–240 g) were used. After pentobarbital anaesthesia (60 mg/kg b.wt. i.p.) rats were laparotomized and partial hepatectomy (approximately 70%) carried out as previously described [8]. A second set of animals that did not undergo hepatectomy but only liver extrusion was used as a group of sham-operated controls. At the indicated times, animals were killed by decapitation and the liver immediately excised and used for the biochemical analysis detailed below. The piece of liver excised during the hepatectomy was also used for these analysis and considered to be a control at zero time.

2.2. Isolation of liver plasma membrane vesicles

Liver plasma membrane vesicles were partially purified as previously reported [9]. Basically the technique involves a Percoll-density gradient centrifugation step able to separate efficiently plasma membrane from the sinusoidal domain of the hepatocyte. This method has been widely validated in our laboratory and yields good quality plasma membrane retaining most transport capacities. These preparations were used to reproduce our previous observations on Na^+-coupled solute transport [4,5] and to measure Na^+,K^+-ATPase activity.

2.3. Na^+,K^+-ATPase activity

The activity of the Na^+,K^+-ATPase was measured in the plasma membrane preparations by the method of Colas and Maroux [10], which is based upon the determination of the ouabain-inhibitable K^+-dependent phosphatase activity of the pump, using *p*-nitrophenol-phosphate as substrate. The assay was performed at 37°C. The enzymatic activity of the pump is expressed as nmol phosphate/mg protein/min.

2.4. Western blot of the Na^+,K^+-ATPase subunits

The amount of Na^+,K^+-ATPase α_1 and β_1 proteins was determined by Western-blotting using specific polyclonal antibodies (U.B.I., Lake Placid, NY). 20 μg protein of crude membranes from the liver homogenates used for the plasma membrane preparations were routinely run on a 10% SDS-polyacrylamide gel under non-reducing conditions. Proteins were transferred to Immobilon-P membranes (Millipore Corporation, Bedford, MA), blocked for at least 1 h in 5% milk, 0.3% Tween-20 PBS (phosphate buffered-saline) and immediately used for immunoreaction. Goat IgG coupled to horseradish peroxidase was used as a second antibody. Blots were developed using fluorescence emission (ECL, Amerhsam, UK). Expression of both subunits, α_1 and β_1, was quantified by densitometry scanning. Results are given as arbitrary units.

2.5. RNA extraction and Northern blot of the Na^+, K^+-ATPase subunits

Total RNA was extracted from liver by the single-step method of

*Corresponding author. Fax: (34) (3) 4021559.

SSDI 0014-5793(95)00217-0

Chomczynski and Sacchi [11]. RNA (15 μg) was fractionated by electrophoresis trhough a 1% agarose–3% formaldehyde gel in a solution of 20 mM 4-morpholine propane sulfonic acid and 1 mM EDTA, pH 7.4. RNA was transferred to nylon membranes (Hybond-N, Amersham, UK) by capillary action in 20 × SSC (3 M NaCl, 300 mM Na citrate, pH 7.0). Efficiency of transfer and control of loading were assessed thanks to ethidium bromide staining of the agarose gels. RNA was crosslinked by UV irradiation. Prehybridization of the blots was carried out for 3 h at 42°C in a medium containing 45% deionized formamide, 4 × SSC, 100 mM sodium phosphate buffer pH 7, 0.1% sodium pyrophosphate, 0.1% SDS, 5 × Denhardt's and 100 μg/ml of purified salmon sperm DNA. Filters were hybridized for 24 h at 42°C in a similar medium, supplemented with 10% dextran sulphate and 1 × Denhardt's. Blots were washed twice at 55°C with a solution of 1 × SSC and 0.1% SDS and one more time with 0.1 × SSC and 0.1% SDS, before autoradiography. Washing of the blots was done at high stringency. cDNA probes used in this study were a 1.2 kb *Eco*RI cDNA fragment from plasmid pRB5.1 for α_1 subunit, and a 1.2 kb *Eco*RI cDNA fragment from plasmid 19G for β_1 mRNA. Both cDNA probes were kindly provided by Dr. Robert Levenson (Yale University School of Medicine). Specific hybridization to the α_1 and β_1 transcripts was quantitated with an AMBIS apparatus. Results are expressed as arbitrary units and normalized to the ethidium bromide staining of the ribosomal bands.

3. Results

3.1. Na^+-coupled solute transport and Na^+,K^+-ATPase activity in plasma membrane vesicles from regenerating liver

Na^+-coupled uptake of alanine and uridine was monitored in plasma membrane vesicles from 6 h regenerating livers as a control to verify the well-known induction of these transport activities in the pre-replicative phase of liver growth. All membrane preparations showed homogeneous inductions (not shown) and were used for the determination of Na^+,K^+-ATPase activity. These results are shown in Table 1. Surgery by itself did not modify significantly the basal enzyme activity of the pump, but the hepatectomy triggered a marked induction of the Na^+, K^+-ATPase activity, already significant 6 h after surgery.

3.2. Na^+,K^+-ATPase subunit proteins

The results of the Western blot analysis of the alpha1 and beta1 subunits of the pump are shown in Fig. 1. The amount of α_1 protein is markedly induced in the regenerating rat liver. This change is already apparent 6 h after surgery. Indeed, the expression of α_1 protein appears to decrease after 6 h in the sham-operated animals. The highest amount of α_1 protein is found 9 h after hepatectomy, when it reaches nearly a four-fold induction above the levels found at time zero. β_1 protein is in the limit of detection of our method and, indeed, in a previous report [12] we were unable to detect this protein using the same conditions and antibodies. By overexposing the films a faint band appears with the expected molecular weight of β_1. The amount of this subunit increases after surgery in the hepatectomized animals. The difference appears to be also significant at the first time tested (6 h). As reported for α_1, the amount of β_1 protein also decreases in the sham-operated animals. The induction of β_1 protein is much less important than that of the α_1 subunit.

Table 1
Na^+,K^+-ATPase activity in plasma membranes from regenerating rat liver

	Na^+,K^+-ATPase activity (nmol P_i/min/mg protein)	
Basal activity (0 time)	7.8 ± 0.5	
Time after surgery	Sham-operated	Hepatectomized
6h	6.6 ± 0.9	10.2 ± 1.0*
9h	7.5 ± 1.2	15.8 ± 3.1*
12h	7.4 ± 1.6	23.3 ± 2.9***

Na^+,K^+-ATPase activity was determined as indicated in section 2, in at least three independent plasma membrane preparations made of three regenerating livers each. Measurements on sham-operated rats were done using four to five independent intact livers. Results are the mean ± S.E.M. Statistical comparisons between the preparations from hepatectomized rats and their respective sham-operated controls were made by the Student's *t*-test (*$P < 0.05$; ***$P < 0.001$).

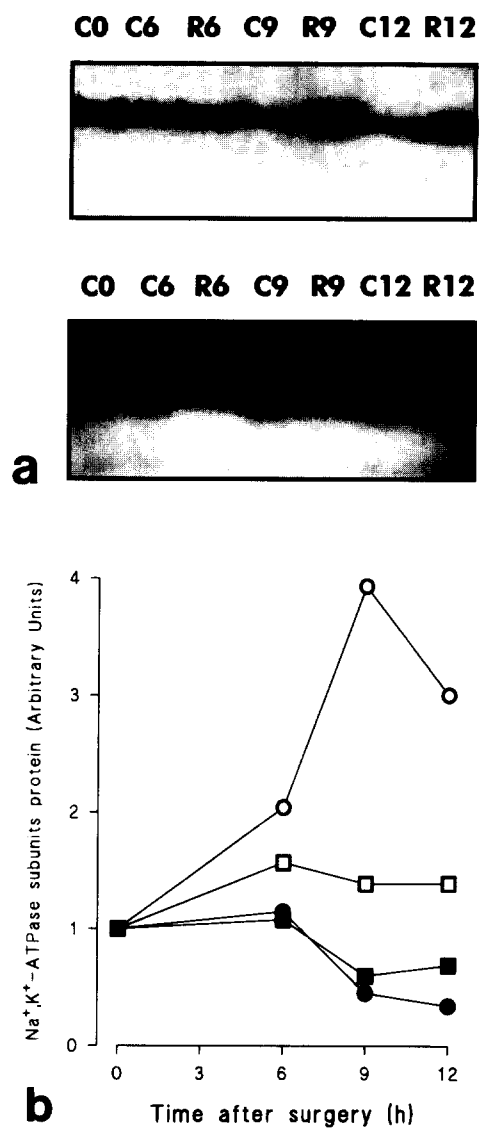

Fig. 1. α_1 and β_1 subunit protein in the regenerating rat liver. A Western blot analysis of both subunits is shown in (a). Upper pannel: 60–200 kDa section of α_1 protein blot; lower pannel: 20–90 kDa section of a β_1 protein blot. C and R correspond to sham-operated and hepatectomized animals respectively. Numbers show the time after hepatectomy in hours. This observation was repeated at least five times using independent plasma membrane preparations and tissue homogenates. Densitometric quantitation of the spots is shown in b. Open symbols correspond to the hepatectomized animals and solid symbols represent the results of the sham-operated rats. Squares for β_1 subunit and circles for α_1 subunit. Experimental details are given in the text.

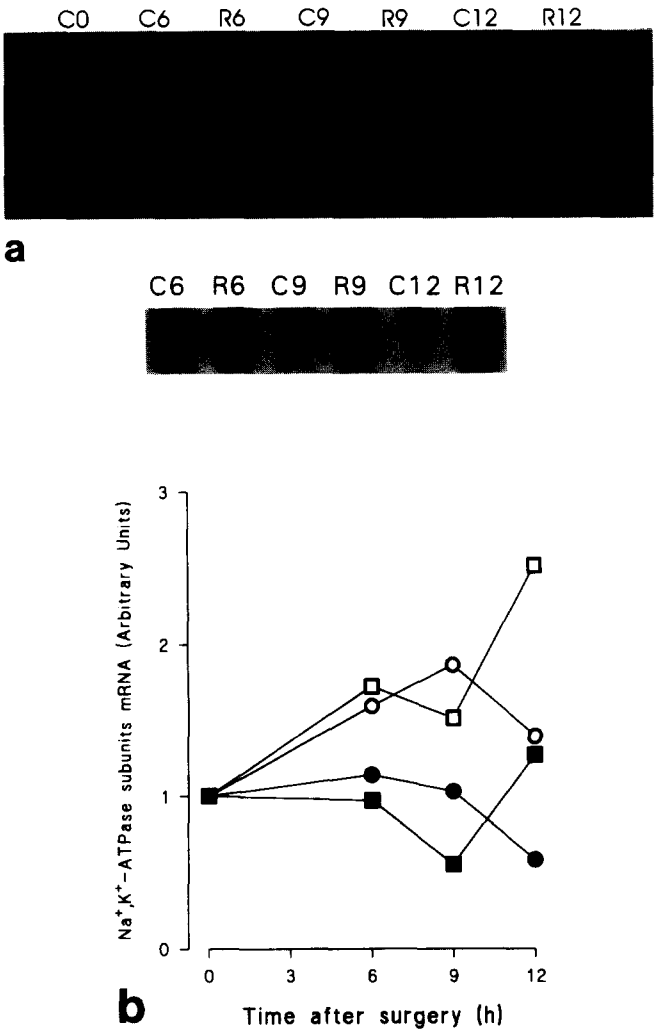

Fig. 2. α_1 and β_1 mRNA levels during rat liver regeneration. A Northern blot analysis of both subunits is shown in (a). Upper pannel: α_1 protein; lower pannel: β_1 protein. C and R correspond to sham-operated and hepatectomized animals, respectively. Numbers show the time after hepatectomy. This observation was repeated at least four times using independent RNA preparations. Densitometric quantitation of the spots is shown in (b). Open symbols correspond to the hepatectomized animals and solid symbols represent the results of the sham-operated rats. Squares for β_1 subunit mRNA and circles for α_1 subunit mRNA. Experimental details are given in the text.

3.3. Na^+,K^+-ATPase subunit mRNAs

The results of the Northern blot analysis of α_1 and β_1 subunit mRNAs are shown in Fig. 2. The amount of mRNA for both subunits is not altered or even decreases after surgery in the sham-operated rats. Nevertheless, in the hepatectomized animals, both messenger RNAs increase and show high levels already 6 h after surgery. Thereafter, their amount appears to increase (β_1) or rather decrease (α_1). In general terms, the pattern of changes of mRNA levels closely ressemble the pattern followed by the expression of subunit proteins, although, quantitatively, this relationship is not so clear.

4. Discussion

This study shows that, in the pre-replicative phase of liver regeneration after partial hepatectomy, the induction of Na^+,K^+-ATPase activity correlates with an increased amount of both subunits of the pump, α_1 and β_1, as well as with accumulation of the mRNAs coding for these proteins. These observations prove that, as suggested for other transporters [4,5], the effect triggered by partial hepatectomy on the activity of the pump relies on a stable change at the plasma membrane involving de novo synthesis of transporters.

Induction of sodium pump activity during the proliferation of liver parenchymal cells has been also described in the perinatal transition [13]. Increased amounts of mRNA for both subunits in liver have been described during postnatal development [14]. Fetal and neonatal hepatocytes also show increased activities of a high affinity Na^+-dependent amino acid transport system, likely to correspond to system A for neutral amino acid uptake [13,15]. Thus, some parallelism exists between both situations, development and liver regeneration. Nevertheless, the mechanisms underlying these adaptations in fetal and regenerating hepatocytes may not be the same according to their differential proliferative response to growth factors and hormones [16].

Some aspects of the regulation of the pump in liver have been established using in vivo models. T_3 administration to hypothyroid rats induces an increase in liver Na^+,K^+-ATPase activity, which is accompanied by an increase in mRNA levels of the α_1 subunit, without significant changes in the amount of β_1 mRNA [17]. The transcription rates for both genes in liver nuclei are however induced by T_3 administration [17], which overall suggests that the pump is under both transcriptional and post-transcriptional control in liver parenchymal cells. Hepatocytes induced to proliferate show increased mRNA levels for the β_1 subunit [7]. This effect is mimicked in primary cultures by a known mitogenic growth factor for liver parenchymal cells, TGFalpha [7]. Indeed, it has been suggested that an increase in β_1 subunit gene expression is somehow related to the growth-associated increases in Na^+,K^+-ATPase activity. In this report, the amounts of protein and mRNA of both subunits are increased showing similar profiles, although they do not keep a close proportionality, since, for instance, the alpha1-subunit protein/mRNA ratio is increased up to two times during liver regeneration. This lack of proportionality is also found when comparing the amounts of α_1 and β_1 subunits and the activity of the pump. These results are consistent with a complex regulation of the expression of the pump, involving either transcriptional, translational or post-translational steps, as has been suggested in the models indicated above and when studying the liver cell line clone 9 [18,19].

Although the combined action of growth factors could be responsible for the increase in the expression of both subunits, there is another key factor to be considered, this is the intracellular sodium concentration. Significant increases in intracellular Na^+ have been reported early after partial hepatectomy [1,2]. This is considered to exert a prompt increase in Na^+, K^+-ATPase activity by means of direct energetization of the pump [2], but, simultaneously, it may induce the transcription of the genes coding for both subunits. A direct effect of Na^+ ions on the transcription rate of the alpha subunit genes has been recently reported [20]. Nevertheless, some evidence obtained from mammalian cell lines suggests that the expression of the sodium pump and that of Na^+-dependent amino acid transport systems (essentially system A) may be coordinately

regulated by nutrient availability (amino acids) even when intracellular Na^+ is low [21,22]. At the present time it is not clear to what extent this may be relevant in vivo and, in particular, in liver regeneration. Nevertheless, other physiological models associated to liver hypertrophia, such as the obese Zucker rat, are also characterized by coordinate induction of Na^+,K^+-ATPase and other Na^+-dependent transport systems, such as those able to take up amino acids and nucleosides [12].

In summary, this work shows that in order to sustain the metabolic demands associated with increased activity of Na^+-coupled solute uptake during the early phase of liver regeneration, the Na^+,K^+-ATPase activity is increased in liver parenchymal cells by a stable mechanism which involves de novo synthesis of new pumps and is accompanied by higher expression of mRNAs for both subunits.

Acknowledgements: We are grateful to Dr. Robert Levenson (Yale University School of Medicine) for kindly providing us with cDNA probes for α_1 and β_1 subunits. This work was supported by Grants PB92-0867, from the Dirección General de Investigación Científica y Técnica (Ministerio de Educación y Ciencia, Spain), and 93/0434 from the Fondo de Investigaciones Sanitarias de la Seguridad Social (Ministerio de Sanidad y Consumo, Spain).

References

[1] Koch, K.S. and Leffert, H.L. (1979) Cell 18, 153–163.
[2] Leffert, H.L., Koch, K.S., Lad, P.J., Shapiro, I.P., Skelly, H. and de Hemptine, B. (1988) in: The Liver: Biology and Pathobiology (Arias, I.M., Jakoby, W.B., Popper, H., Schachter, D. and Shafritz, D.A. Eds.) 2nd edn., pp. 833–850, Raven Press, New York.
[3] Fowler, F.C., Banks, R.K. and Mailliard, M.E. (1992) Hepatology 16, 1187–1194.
[4] Martínez-Mas, J.V., Ruiz-Montasell, B., Felipe, A., Casado, J. and Pastor-Anglada, M. (1993) FEBS Lett. 329, 189–193.
[5] Ruiz-Montasell, B., Martínez-Mas, J.V., Enrich, C., Casado, F.J., Felipe, A. and Pastor-Anglada, M. (1993) FEBS Lett. 316, 85–88.
[6] Enrich, C., Bachs, O., Soriano, M. and Serratosa, J. (1986) Biochim. Biophys. Acta 861, 381–384.
[7] Lu, X.P. and Leffert, H.L. (1991) J. Biol. Chem. 266, 9276–9284.
[8] Higgins, G.F. and Anderson, R.M. (1931) Arch. Pathol. 12, 186–202.
[9] Pastor-Anglada, M., Remesar, X. and Bourdel, G. (1987) Am. J. Physiol. 252, E408–E413.
[10] Colas, B. and Maroux, S. (1980) Biochim. Biophys. Acta 600, 406–420.
[11] Chomczynski, K. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[12] Ruiz-Montasell, B., Ferrer-Martínez, A., Casado, F.J., Felipe, A. and Pastor-Anglada, M. (1994) Biochim. Biophys. Acta, in press.
[13] Bellemann, P. (1981) Biochem. J. 198, 475–483.
[14] Emanuel, J.R., Garetz, S., Stone, L. and Levenson, R. (1987) Proc. Natl. Acad. Sci. USA 84, 9030–9034.
[15] Martínez-Mas, J.V., Casado, J., Felipe, A., Marín, J.J.G. and Pastor-Anglada, M. (1993) Biochem. J. 293, 819–824.
[16] De Juan, C., Benito, M., Alvarez, A. and Fabregat, I. (1992) Exp. Cell Res. 202, 495–500.
[17] Gick, G.G., Ismail-Beigi, F. and Edelman, I.S. (1988) J. Biol. Chem. 263, 16610–16618.
[18] Pérez, C., Bhutada, A. and Ismail-Beigi, F. (1991) Am. J. Physiol. 260, C417–C423.
[19] Buthada, A., Wassynger, W.W. and Ismail-Beigi, F. (1991) J. Biol. Chem. 266, 10859–10866.
[20] Yamamoto, K., Ikeda, U., Seino, Y., Tsuruya, Y., Oguchi, A., Okada, K., Ishikawa, S., Saito, T., Kawakami, K., Hara, Y. and Shimada, K. (1993) J. Clin. Invest. 92, 1889–1895.
[21] Qian, N.X., Pastor-Anglada, M. and Englesberg, E. (1991) Proc. Natl. Acad. Sci. USA 86, 7984–7988.
[22] McGivan, J.D. and Pastor-Anglada, M. (1994) Biochem. J. 299, 321–334.