ELSEVIER

Biochimica et Biophysica Acta 1196 (1994) 45–50

BBA Biochimica et Biophysica Acta

Coordinate induction of Na^+-dependent transport systems and Na^+,K^+-ATPase in the liver of obese Zucker rats

Bonaventura Ruiz-Montasell, Andreu Ferrer-Martinez, Francisco Javier Casado, Antonio Felipe, Marçal Pastor-Anglada *

Unitat de Bioquímica i Biologia Molecular B, Departament de Bioquímica i Fisiologia, Universitat de Barcelona, Facultat de Biologia, Diagonal 645, 08028 Barcelona, Spain

Received 25 March 1994; revised 12 July 1994

Abstract

Solute uptake into liver plasma membrane vesicles from either lean or obese Zucker rats was monitored. D-Glucose and L-leucine uptakes at physiological concentrations of the substrate were not different in lean and obese Zucker rats. In agreement with a previous report (Ruiz et al. (1991) Biochem. J. 280, 367–372) L-alanine uptake was significantly enhanced in those preparations from obese animals. Na^+-coupled uridine transport was markedly enhanced also in obese rats. The effect was due to an increase in V_{max} (5.5 ± 0.6 vs. 2.1 ± 0.2 pmol/mg protein per 3 s, $P < 0.01$) without any significant change in K_m (11.0 ± 2.8 vs. 9.0 ± 2.7 μM for obese and lean rats, respectively). Na^+,K^+-ATPase activity was also higher in liver plasma membrane vesicles from rat liver and it correlated with a higher amount of α_1- subunit protein in both, plasma membrane vesicles and homogenates from obese rat livers. In summary, in the hypertrophic liver of obese Zucker rats a coordinate induction of several Na^+-dependent transport systems occurs and, in order to sustain the metabolic pressure associated with this adaptation, a significant induction of the Na^+,K^+-ATPase expression is also found. These data also provide new evidence for regulation of the recently characterized Na^+-dependent nucleoside transporter.

Keywords: Sodium ion dependent transport; Nucleoside uptake; ATPase, Na^+/K^+-; Liver plasma membrane vesicle; (Zucker rat)

1. Introduction

The liver shows the almost unique property of undergoing hypertrophic and/or hyperplasic growth even in the adult state. Some physiological and pathophysiological situations associated with liver growth are pregnancy [1], lactation [1], feeding on hyperproteic diets [2], liver regeneration after partial hepatectomy [3] and genetic obesity [4]. To sustain liver growth, substrates for nucleic acid and protein synthesis are required. All the conditions cited above are indeed characterized by enhanced activity of most Na^+-dependent amino acid transport systems at the plasma membrane level [3,5–9].

The Zucker rat, a model extensively used in the study of human obesity, is also characterized by early liver hypertrophy due to enhanced protein accretion [4,8,10]. In fact, a higher liver mass is characteristic of young obese animals long before steatosis appears [8]. In older animals, even liver DNA content is higher in obese than in lean rats [10]. In a previous study from our laboratory [8] we reported the coordinate induction of several Na^+-dependent amino acid transport systems in liver plasma membrane vesicles from young (7–8-week old) obese Zucker rats. Systems A, ASC and N were significantly induced by a mechanism involving a V_{max} effect (2-fold over control values), without significantly altering the K_m. Thus, the metabolic activity of liver parenchymal cells from obese rats may be enhanced, since active transport of solutes across the plasma membrane would probably determine a compensative increase in Na^+,K^+-ATPase expression. This work was aimed first, to study whether other Na^+-coupled solute transporters showed increased activities in this model, and, second, to determine whether the metabolic pressure of these adaptations was high enough as to induce a higher expression of the sodium pump. As another Na^+-dependent transport system likely to be up-regulated in this model, we chose the Na^+-dependent nucleoside transporter recently characterized in our laboratory [11]. This transport system mediates the concentrative uptake of

* Corresponding author. Fax: +34 3 4021559.

SSDI 0005-2736(94)00202-9

most nucleosides, shows a stoichiometry of 1 nucleoside/1 Na^+ and is sensitive to membrane potential. Little is known on the regulation of this transport system, but its activity is markedly induced soon after partial hepatectomy [12]. In the present report we show that the concentrative nucleoside transporter is induced in the obese Zucker rat, as are the Na^+-dependent amino acid transport systems. This further supports the view that concentrative nucleoside transport in liver parenchymal cells is somehow regulated, and opens the possibility that in liver hypertrophia a coordinate effect on Na^+-dependent transporters occurs, because glucose and leucine uptakes were not significantly modified. As hypothesized, this induction correlated with increased Na^+,K^+-ATPase expression.

2. Materials and methods

2.1. Animals

Six-week old obese Zucker rats were purchased from Iffa-Credo (Barcelona, Spain) and kept under controlled conditions of temperature (22 ± 2°C), humidity (40–60%) and light (12 h on/12 h off) until they were 50–52-day old, when they were used for the experiments. Mean body weights of lean and obese Zucker rats were 185 and 240 g, respectively. These values showed little variability (less than 3% S.E.) and allowed us to define two independent populations of animals. Previous experiments leading to the present study were performed at the same stage [8].

2.2. Preparation of plasma membrane vesicles from rat liver

Plasma membrane vesicles were purified from the liver of single animals by a Percoll density-gradient method, previously described and widely used, and validated in our laboratory [7–9,13–15]. The protein content of the preparations was measured according to Peterson [16]. The contamination by subcellular membranes other than the plasma membrane was assessed by measuring the activity of several enzyme markers, as previously described [6]. The enzyme pattern of the preparations used was similar to that reported in our previous study using obese Zucker rats [8], and showed very low contamination by other membranes and selective enrichment of plasma membrane. This was also verified when studying Na^+,K^+-ATPase expression in homogenates and vesicle preparations (Fig. 3).

2.3. Uptake measurements in plasma membrane vesicle preparations

Solute transport (L-alanine, L-leucine, D-glucose and uridine) into liver plasma membrane vesicles was measured by a filtration method adapted from Sips et al. [17] as described in Pastor-Anglada et al. [6]. The incubation medium consisted of 0.25 M sucrose, 0.2 mM $CaCl_2$, 10 mM $MgCl_2$, 10 mM Hepes-KOH (pH 7.4), 100 mM NaSCN or KSCN and the substrate of interest. L-[2,3-^{3}H]Alanine was used at a concentration of 0.25 mM at a specific activity of 45 μCi/μmol. L-[^{3}H]Leucine at a concentration of 0.25 mM and at a specific activity of 90 μCi/μmol. Transport of 5 mM D-[U-^{14}C]glucose was measured using a specific activity of 7 μCi/μmol. Uridine uptake was determined in a range of solute concentrations from 0.1 up to 50 μM, with specific activities ranging from 6.8 to 680 μCi/μmol. The uptake experiments were initiated by mixing 20 μl of the incubation medium with 20 μl of plasma membrane preparations (around 160 μg of protein). The measurements were stopped at the indicated times by adding 1 ml of an ice-cold stop solution consisting of 0.25 M sucrose, 100 mM NaCl, 0.2 mM $CaCl_2$, 10 mM Hepes-KOH at pH 7.4. The whole volume was then filtered through nitrocellulose filters (0.45 μm pore size) (Schleicher and Schüell, Germany), then the filters were washed with 4 ml of the ice-cold buffer and later counted for radioactivity. Transport rates were calculated according to the specific activity of the substrate and expressed either as nmol substrate/mg protein (L-alanine, L-leucine and D-glucose) or as pmol/mg protein (uridine).

2.4. Calculation of the kinetic parameters

Kinetic parameters of the Na^+-dependent uptake of uridine were derived mathematically from the experimental data by non-linear regression analysis using the Enzfitter software (Elsevier Biosoft, Cambridge, UK).

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

The activity of Na^+,K^+-ATPase was measured in both homogenates and liver plasma membrane vesicles from both lean and obese Zucker rats, by the method of Colas and Maroux [18]. This method is based upon the determination of the ouabain-inhibitable K^+-dependent phosphatase activity of the pump, using *p*-nitrophenol phosphate as substrate. Briefly, 50 μl of sample (1 mg protein/ml) were preincubated for 30 min at 37°C in 0.5 ml of a medium containing 50 mM Tris base, 10 mM $MgSO_4 \cdot 7H_20$, 5 mM EDTA and 90 mM KCl (pH 7.6), either in the presence or the absence of saturating concentrations of ouabain (0.7 mM). Then 0.5 ml of the same medium supplemented with 12 mM *p*-nitrophenol phosphate was added and incubated for a further 30 min. The reaction was stopped by adding 0.2 ml of 30% trichloroacetic acid. The whole volume of the tube was centrifuged for 5 min in a microfuge at maximum speed (14 000 rpm) and the supernatants were transferred to other tubes and neutralized with 2 ml of 1 M Tris base. The *p*-nitrophenol produced was measured with a spectrophotometer as absorbance at 410 nm. A standard of *p*-nitrophenol was used to convert the

spectrophotometric data into nmol phosphate/mg protein per min. The activity in the homogenate fractions was below the sensitivity of the method and only the results obtained in plasma membrane vesicle preparations are shown.

2.6. Na^+,K^+-ATPase α_1-subunit expression

The amount of Na^+,K^+-ATPase α_1-protein was determined by Western-blotting using specific polyclonal antibodies (U.B.I., Lake Placid, NY). 64 μg protein from homogenates and 20 μg protein from plasma membrane vesicles were routinely run on a 10% SDS-polyacrylamide gel, under the conditions specified by the manufacturer. Proteins were transferred into hydrophobic filters (Immobilon-P, Millipore, Bedford, MA), blocked in 5% milk, 0.3% Tween-20 PBS and immediately used for immunoreaction. Goat IgG coupled to horseradish peroxidase was used as second antibody. Blots were developed either using diaminobenzidine or fluorescence emission (ECL, Amersham, UK). α_1-subunit expression was quantified by densitometry scanning of the spots. The results are given as arbitrary units. Expression of the β_1-isoform could not be detected with the available antibodies in these preparations.

3. Results

3.1. Time-course of solute uptake into liver plasma membrane vesicles

The time-courses of solute uptake into plasma membrane vesicles from livers of lean and obese Zucker rats are shown in Fig. 1. L-Alanine and uridine were taken up by these preparations in a concentrative and Na^+-dependent manner. Uptake rates measured in a NaSCN medium were much higher than in a KSCN medium. Furthermore, both substrates were more actively transported into those vesicles from obese Zucker rats than into those from their lean littermates. D-Glucose and L-leucine uptake rates were not significantly different in NaSCN and KSCN media, as expected, considering both solutes are transported almost exclusively by facilitated transport systems in liver parenchymal cells. No significant differences were found for these two substrates between preparations from obese and lean Zucker rats.

3.2. Kinetics of Na^+-dependent uridine uptake

As shown in Fig. 1, uridine uptake into plasma membrane vesicles was very rapid. Thus, a 3-s incubation time (initial velocity conditions) was chosen to perform the kinetic studies. Na^+-dependent uptake was calculated after substracting the uptake rates measured in a KSCN medium from those determined in a NaSCN medium. The dependence of the Na^+-coupled uridine uptake on the substrate concentration follows the classical Michaelis-Menten equation and is shown in Fig. 2. From these experimental data we calculated the following kinetic parameters. V_{max} values were 5.5 ± 0.6 and 2.1 ± 0.2 pmol/mg protein per 3 s for obese and lean Zucker rats, respectively. This difference was statistically significant ($P < 0.01$). The K_m values remained unaltered: 11.0 ± 2.8 and 9.0 ± 2.7 μM for obese and lean rat liver preparations, respectively.

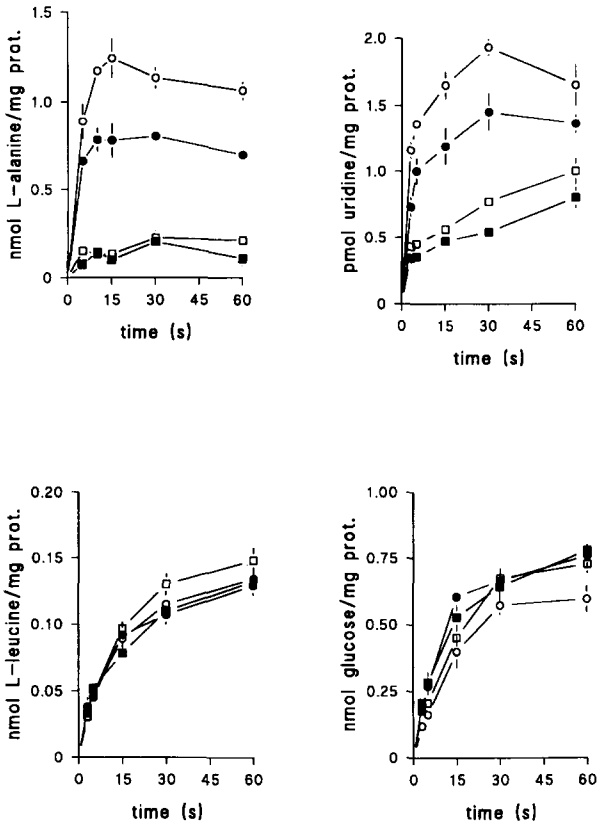

Fig. 1. Uptake of organic solutes into liver plasma membrane vesicles from lean and obese Zucker rats. Liver plasma membrane vesicles either from lean (solid symbols) or from obese (open symbols) Zucker rats were incubated either in a NaSCN (round symbols) or in a KSCN (square symbols) medium, in the presence of 0.25 mM L-alanine, 0.25 mM L-leucine, 5 mM D-glucose or 1 μM uridine. The substrate retained by the vesicles was determined, as explained in Methods, at the indicated times. The results are the means ± S.E. of triplicate observations made on pools from at least four independent preparations. Significant differences between obese and lean rats were only found when measuring L-alanine and uridine uptake in the NaSCN medium. Where no error bars appear they are included in the symbols.

3.3. Activity and expression of Na^+,K^+-ATPase

Expression of Na^+,K^+-ATPase α_1-subunit in homogenates and vesicles (Fig. 3a) gives an estimation of the enrichment in plasma membrane achieved in our preparations. The densitometric analysis of this blot gave nearly three times more protein in the vesicle than in the homogenate. However, considering that three times more

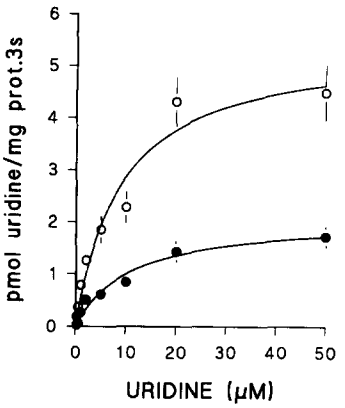

Fig. 2. Na^+-dependent uridine uptake as a function of substrate concentration in liver plasma membrane vesicles from lean and obese Zucker rats. Na^+-dependent uridine uptake, calculated as the difference between the uptake rates in NaSCN and in KSCN media, are shown as a function of substrate concentration. The data followed classical Michaelis-Menten kinetics and were fitted according to a non-linear regression analysis, to give the kinetic parameters indicated in the text. The results are the mean of triplicate observations made on five independent preparations from lean and obese Zucker rats. Statistical significance ($P < 0.05$) was found for each substrate concentration tested.

homogenate protein was loaded in the gel, this gave a mean α_1-subunit enrichment of 9-fold, which is in agreement with previous preparations in which other markers had been used [6]. The enrichment was quite homogeneous between preparations and not significantly different between both experimental groups. When comparing the amount of α_1-subunit protein in homogenate and plasma membrane vesicles between preparations from obese and lean rats (Figs. 3b and 3c), it was evident that the amount of protein was double no matter what sample was used, homogenate or vesicles. This was associated with a significant increase in Na^+,K^+-ATPase activity measured in the vesicle preparations. Nevertheless, the increase in the biological activity of the pump was barely 50% over the values found in those preparations from lean rats.

4. Discussion

4.1. Induction of Na^+-dependent transport systems in the liver of obese Zucker rats

As indicated above, at this stage of development of the obese phenotype and in association with liver hypertrophy, the Na^+-dependent amino acid transport systems A, ASC and N are significantly induced [8]. So far, there is no clear explanation for this phenomenon. A first possibility is gene programming, based on the pleiotropic effects described in cultured cell lines, where coordinate induction of more than one Na^+-dependent amino acid transport system is observed [19,20]. This pleiotropism does not affect the non-concentrative transport system L. Indeed, this is also the case in the present model, where L-leucine uptake (mostly by system L) is not altered in the obese phenotype. Another possibility is that these transport systems may be under coordinate hormonal control. Nevertheless, only systems A and N are known to be sensitive to hormone action (for review, see [21–23]), although system ASC is also up-regulated in a few pathophysiological models, including streptozotocin-induced diabetes [24]. The biochemical basis for the induction of Na^+-dependent uridine transport is unknown. Since this transport system has been characterized only very recently [11], nothing is known about its regulation. As indicated above, the transporter is induced early after partial hepatectomy, during the prereplicative phase of liver regeneration [12]. It could be assumed that

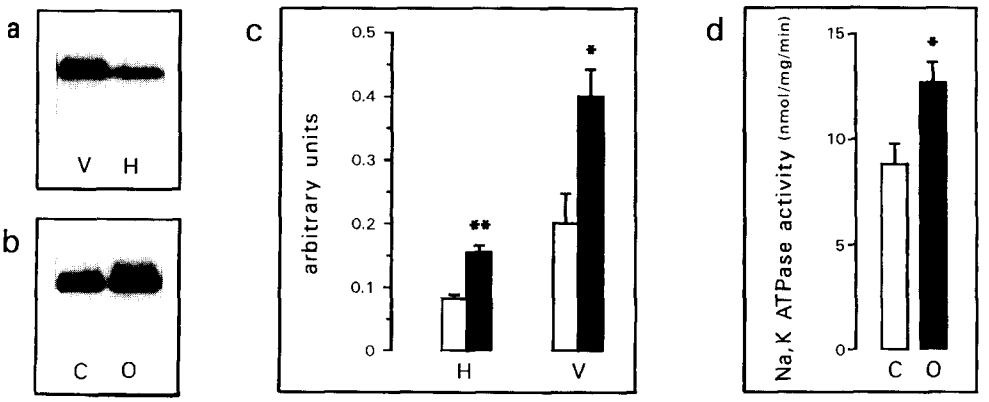

Fig. 3. Expression of Na^+,K^+-ATPase in the liver of lean and obese Zucker rats. A representative Western blot analysis of liver homogenate (64 μg protein) (H) and plasma membrane vesicles (20 μg protein) (V) from control lean Zucker rats showed that a substantial enrichment of Na^+,K^+-ATPase α_1-subunit was achieved in the vesicle preparations (Fig. 3a). The densitometry of five independent preparations from such animals is shown in Fig. 3c (open bars). A representative result of a Western blot of Na^+,K^+-ATPase α_1-subunit in plasma membrane vesicles from obese (O) and lean (C) rats is shown in Fig. 3b. The densitometry of five independent preparations from each experimental group is shown in Fig. 3c (open bars, lean; solid bars, obese). The biological activity of the Na^+,K^+-ATPase in liver plasma membrane vesicles from either obese (O) or lean (C) Zucker rats is shown in Fig. 3d. These results are the means ± S.E. of quadruplicate observations made on five independent preparations. Statistical significance of the differences was assessed using the Student's t-test ($^*P < 0.05$; $^{**}P < 0.01$).

those factors involved in the proliferative response of the liver could be potential regulators of this transport activity. This aspect is currently under research and, so far, evidence for long-term regulation of this transport system by insulin has been found, either using primary cultures of rat hepatocytes or liver plasma membrane vesicles from euglycemic hyperinsulinemic rats (unpublished data). The present report, showing up-regulation of Na^+-dependent uridine transport in the obese phenotype, offers further evidence in favor of the view that this transport system may be under hormonal control. Since obese Zucker rats are already hyperinsulinemic [25], a putative role of insulin may well underlie on the induction of the nucleoside transporter. Other possibilities may be too speculative, but one of them merits discussion. Pleiotropy in the induction of Na^+-dependent amino acid transport systems in CHO-K1 cells was attributed to a putative regulatory or structural subunit acting on several transporters at a time [19,20]. No molecular evidence has been provided so far, basically because most of these transport systems have not yet been characterized at the molecular level. A putative system A cDNA was recently isolated by Kong et al. [26]. It showed high homology with the Na^+/glucose cotransporter cloned by Wright and co-workers [27]. A cDNA encoding a putative renal Na^+-dependent nucleoside transporter has also been recently isolated and shown to belong to the same family [28]. Nevertheless, this cDNA is not expressed in liver and there is no evidence that the hepatic transporter shows homology with this group of plasma membrane proteins. In any case, the recent finding by Koepsell and co-workers [29] that a regulatory subunit, RS1, could activate and modulate the intestinal Na^+/glucose cotransporter, as well as other members of the same family, like the Na^+/*myo*-inositol cotransporter, opens the possibility that a single protein or a group of highly homologous regulatory proteins may coordinately modulate the activity of several Na^+-dependent transporters. Our laboratory has also provided recent evidence of a putative regulatory protein involved in the up-regulation of system A activity after hypertonic shock [30,31]. This possibility, although perhaps speculative, should be kept in mind in the future when analyzing those situations characterized by coordinate induction of several Na^+-dependent transporters.

4.2. Induction of Na^+,K^+-ATPase

Induction of most Na^+-dependent solute transporters must be supported by higher efficiency in Na^+ pumping. We show here that induction of Na^+,K^+-ATPase activity occurs in the hypertrophic liver of obese Zucker rats. Evidence for coordinate regulation between system A and Na^+,K^+-ATPase has been recently reviewed [23]. In particular, coordinate induction of system A and the Na^+,K^+-ATPase α_1-subunit gene has been proved in CHO-K1 cells and alanine-resistant mutants [32,33]. The possibility that hormones are modulating Na^+,K^+-ATPase in liver parenchymal cells cannot be ruled out. Most of the regulation of Na^+,K^+-ATPase gene expression in liver has been studied using a non-transformed liver cell line, clone 9. Abundance of mRNA for both α_1- and β_1-subunits is modulated by a variety of factors, serum, dexamethasone, cycloheximide, insulin and most growth-factors [34–37]. However, it never resulted in a net increase in enzyme activity, which suggests strong translational and/or post-translational control. In the hypertrophic liver of obese Zucker rats enhanced expression of the α_1-subunit protein occurs. In this case, it seems to be associated with increased mRNA α_1 levels (not shown). The lack of correspondence between enzyme induction (40% above basal values) and α_1-protein accumulation (100% above basal values) may be explained either by post-translation modification or by limitation due to low β_1 synthesis compared to α_1. Since both isoforms are differentially regulated, the possibility that one subunit is synthesized in molar excess over the other is not unlikely and has been reported in other cell models [38,39]. The lack of immunoreactivity on the β-subunit of the antibodies used does not allow direct proof of this hypothesis.

In conclusion the hypertrophic liver of obese Zucker rats is a physiological model of coordinate induction of Na^+-dependent transporters and the Na^+,K^+-ATPase. This adaptation probably contributes to protein accretion and active cellular metabolism, by increasing the availability of key substrates for protein and nucleic acid biosynthesis. This study also provides further evidence for regulation of the Na^+-dependent nucleoside transport system recently characterized in liver plasma membrane vesicles.

Acknowledgements

This work has been supported by grants PB92-0867 from the D.G.I.C.Y.T. (Ministerio de Educación y Ciencia, Spain) and 93/0434 from the F.I.S. (Ministerio de Sanidad y Consumo, Spain). A.F.-M. is a predoctoral Fellow from F.I.S.

References

[1] Remesar, X., Arola, Ll., Palou, A. and Alemany, M. (1981) Lab. Anim. Sci. 31, 67–70.
[2] Rémésy, C. and Demigné, C. (1982) J. Nutr. 112, 60–69.
[3] Leffert, H.L., Koch, K.S., Lad, P.J., Shapiro, I.P., Skelly, H. and De Heptine, 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.
[4] Cleary, M.P., Vasselli, J.R. and Greenwood, M.R.C. (1980) Am. J. Physiol. 238, E284–E292.
[5] Fafournoux, P., Remésy, C. and Demigné, C. (1982) Biochem. J. 206, 13–18.
[6] Pastor-Anglada, M., Remesar, X. and Bourdel, G. (1987) Am. J. Physiol. 252, E408–E413.

[7] Felipe, A., Remesar, X. and Pastor-Anglada, M. (1989) Pediatr. Res. 26, 448–451.
[8] Ruiz, B., Felipe, A., Casado, J. and Pastor-Anglada, M. (1991) Biochem. J. 280, 367–372.
[9] Martínez-Mas, J.V., Ruiz-Montasell, B., Felipe, A., Casado, J. and Pastor-Anglada, M. (1993) FEBS Lett. 329, 189–193.
[10] Greenwood, M.R.C., Maggio, C.A., Koopmans, H.S. and Sclafani, A. (1982) Int. J. Obesity 6, 513–525.
[11] Ruiz-Montasell, B., Casado, F.J., Felipe, A. and Pastor-Anglada, M. (1992) J. Membr. Biol. 128, 227–233.
[12] Ruiz-Montasell, B., Martínez-Mas, J.V., Enrich, C., Casado, F.J., Felipe, A. and Pastor-Anglada, M. (1993) FEBS Lett. 316, 85–88.
[13] Casado, J., Felipe, A., Pastor-Anglada, M. and Remesar, X. (1988) Biochem. J. 256, 377–381.
[14] Quintana, I., Felipe, A., Remesar, X. and Pastor-Anglada, M. (1988) FEBS Lett. 235, 224–228.
[15] Felipe, A., Remesar, X. and Pastor-Anglada, M. (1993) Metab. Clin. Exp. 42, 1109–1115.
[16] Peterson, G.L. (1979) Anal. Biochem. 100, 201–220.
[17] Sips, H.J., Van Amelsvoort, J.M.M. and Van Dam, K. (1980) Eur. J. Biochem. 105, 217–224.
[18] Colas, B. and Maroux, S. (1980) Biochim. Biophys. Acta 600, 406–420.
[19] Moffett, J. and Englesberg, E. (1984) Mol. Cell. Biol. 4, 799–808.
[20] Englesberg, E. and Moffett, J. (1986) J. Membr. Biol. 91, 199–212.
[21] Kilberg, M.S. and Häussinger, D. (1992) Mammalian Amino Acid Transport, Plenum Press, New York.
[22] Kilberg, M.S., Stevens, B.R. and Novak, D.A. (1993) Annu. Rev. Nutr. 13, 137–165.
[23] McGivan, J.D. and Pastor-Anglada, M. (1994) Biochem. J. 299, 321–334.
[24] Barber, E.F., Handlogten, M.E., Vida, T.A. and Kilberg, M.S. (1982) J. Biol. Chem. 257, 14960–14967.
[25] Pénicaud, L., Ferré, P., Terretaz, J., Kinebanyan, M.F., Leturque, A., Doré, E., Girard, J., Jeanrenaud, B. and Picon, L. (1987) Diabetes 36, 626–631.
[26] Kong, C.T., Yet, S.F. and Lever, J.E. (1993) J. Biol. Chem. 268, 1509–1512.
[27] Hediger, M.A., Coady, M.J., Ikada, T.S. and Wright, E.M. (1987) Nature 330, 379–381.
[28] Pajor, A.M. and Wright, E.M. (1992) J. Biol. Chem. 267, 3557–3560.
[29] Vehyl, M., Spangenberg, J., Püschel, B., Poppe, R., Dekel, C., Fritzsch, G., Haase, W. and Koepsell, H. (1993) J. Biol. Chem. 268, 25041–25053.
[30] Gómez-Angelats, M., Ruiz-Montasell, B., Casado, F.J., Felipe, A., McGivan, J.D. and Pastor-Anglada, M. (1994) Biochem. Soc. Trans. 22, 163S.
[31] Ruiz-Montasell, B., Gómez-Angelats, M., Casado, F.J., Felipe, A., McGivan, J.D. and Pastor-Anglada, M. (1994) Proc. Natl. Acad. Sci. USA, in press.
[32] Qian, N.X., Jones, M., McDonough, A. and Englesberg, E. (1989) Proc. Natl. Acad. Sci. USA 86, 7984–7988.
[33] Qian, N.X., Pastor-Anglada, M. and Englesberg, E. (1991) Proc. Natl. Acad. Sci. USA 88, 3416–3420.
[34] Bhutada, A., Perez, C., Chon, D.Y. and Ismail-Beigi, F. (1990) Am. J. Physiol. 258, C1044–C1050.
[35] Bhutada, A. and Ismail-Beigi, F. (1991) Am. J. Physiol. 261, C699–C707.
[36] Perez, C., Bhutada, A. and Ismail-Beigi, F. (1991) Am. J. Physiol. 260, C417–C423.
[37] Bhutada, A., Wassynger, W.W. and Ismail-Beigi, F. (1991) J. Biol. Chem. 266, 10859–10866.
[38] Geering, K., Theulaz, I., Verrey, F., Häuptle, M.T. and Rossier, B.C. (1989) Am. J. Physiol. 257, C851–C858.
[39] Mircheff, A.K., Bowen, J.W., Yiu, S.C. and McDonough, A.A. (1992) Am. J. Physiol. 262, C470–C483.