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Early induction of Na^+-dependent uridine uptake in the regenerating rat liver

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Na^+-dependent uridine transport into liver plasma membrane vesicles from partially hepatectomized and sham-operated rats was studied. Preparations purified 6 h after 70% hepatectomy exhibited an increased V_{max} of uridine uptake (3.7 vs. 1.4 pmol/mg prot/3 s) without any change in K_m (6 μM). Incubation of the vesicles in the presence of monensin decreased uridine uptake although the differences between both experimental groups remained identical. It is concluded that uridine transport is induced early after partial hepatectomy by a mechanism which does not involve changes in the transmembrane Na^+ gradient. This is the first evidence in favor of modulation of nucleoside transport into liver cells.

Nucleoside transport; Liver regeneration; Rat liver

1. INTRODUCTION

Nucleoside transport across the plasma membrane is mediated by different transport systems. So far, two Na^+-independent agencies, differing in their sensitivity to inhibition by nitrobenzylthioinosine (NBMPR), dilazep and dipyridamole have been described [1–7]. In absorptive epithelia, such as those of renal cortex and small intestine, Na^+-dependent uptake has been also reported [4,6,8–12]. Williams and Jarvis [13] suggested that two separate Na^+/nucleoside cotransporters were present in bovine renal brush-border membrane vesicles. They were called N1 and N2 and differed in their specificity for purine and pyrimidine nucleosides. This group has also been able to express N2 activity by injecting mRNA into *Xenopus* oocytes [14]. More recently, Pajor and Wright reported the molecular cloning of a mammalian Na^+/nucleoside cotransporter (SNST1) and other related proteins, which apparently are members of a gene family sharing high homology with the intestinal Na^+/glucose cotransporter [15]. Interestingly, SNST1 mRNA was abundant in outer and mid renal cortex but absent in small intestine and liver. These findings suggest the existence of separate Na^+-dependent nucleoside transporters in mammalian cells. However, Na^+-dependent nucleoside uptake by liver parenchymal cells has been reported and fully characterized very recently [16–18], although it has been known for several years that the liver is especially active metabolizing afferent nucleosides, which probably explains their rapid plasma turnover [19–22]. Recent observations suggest that this carrier is located at the canalicular domain of the hepatocyte plasma membrane and its physiological role would be the conservation of nucleosides generated by canalicular ectonucleotidases [16,17].

In a previous study using preparations of plasma membrane vesicles from rat liver, we were able to characterize uridine transport [18]. The carrier showed a broad specificity and was electrogenic and evidence was provided in favor of a stoichiometry of 1 Na^+/1 uridine. At that time we wondered whether this activity was constitutively expressed in rat liver or it could be somehow regulated in order to increase the provision of nucleosides to liver parenchymal cells when these were induced to proliferate. In this study we tested this hypothesis by measuring uridine uptake in rat liver plasma membrane vesicles from animals that underwent partial hepatectomy.

2. MATERIALS AND METHODS

2.1. *Animals and surgery*

Wistar rats were purchased from the Animal Care facilities of the University of Barcelona. They had been kept under controlled conditions of temperature (22 ± 2°C), humidity (40–60%) and light (12 h light–dark cycle) and fed a standard diet (UAR A04, Panlab, Barcelona, Spain). All animals were starved for 24 h before a 70% hepatectomy was performed. They were anaesthetized with sodium pentobarbital (60 mg/kg b.w. intraperitoneally). The use of other anaesthetics like ketolar or ether did not modify the results (not shown). A set of rats were sham-operated and underwent laparotomy and liver extrusion without hepatectomy. Surgical procedures were as previously described [23] and were carried out at 8.00 h, at the beginning of the

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light cycle. The portion of liver removed from the hepatectomized animals was used for plasma membrane vesicle preparation and characterized for uridine transport. This was taken as a control of basal uptake and called C_0. Hepatectomized and sham-operated rats were sacrificed by decapitation 6 h after surgery and used for liver plasma membrane preparations. They were called R_6 and C_6, respectively.

2.2. *Preparation of plasma membrane vesicles from rat liver*

Plasma membrane vesicles were isolated by the method described in [24]. This procedure involves a Percoll density centrifugation; it has been widely used in our laboratory and the characteristics of the plasma membrane vesicles have been published elsewhere [24–27]. The isolated vesicles were resuspended in 0.25 M sucrose, 0.2 mM $CaCl_2$, 10 mM $MgCl_2$, 10 mM HEPES/KOH (pH 7.4), frozen in liquid nitrogen and stored at −40°C until use. Uridine transport activity in these vesicles was stable for up to 2 months (not shown). The protein content of the preparations was measured according to [28].

To determine to what extent fractions from hepatectomized (R_6) or sham-operated (C_6) rats were comparable, we performed the following assays. First, we analyzed the profile of plasma membrane proteins of C_6 and R_6 samples, by running an SDS-PAGE in 10% acrylamide resolving gels according to [29]. Then, the gels were stained with Coomassie blue and pictures taken. To characterize the presence of membranes from the sinusoidal and canalicular domains of the plasma membrane, we performed Western blotting analysis, as previously reported [30]. Protein AGp110 and the asialoglycoprotein receptor were used as markers of the canalicular and sinusoidal domains respectively. Antiserum against rat AGp110 was raised in rabbits as described in [31] and the affinity-purified antibody to the 42 kDa subunit of the asialoglycoprotein receptor was obtained as reported in [30]. Second, we determined the apparent vesicle volumes of both preparations as follows. We incubated the plasma membrane vesicles for 30 min in the presence of 50 μM L-alanine. This time was long enough in order to reach equilibrium between extra- and intravesicular solute concentrations. Then, we calculated the apparent vesicle volumes by dividing the amount of solute retained by the L-alanine concentration in the medium. Apparent volumes were 460 ± 16 and 492 ± 26 nl/mg protein for C_6 and R_6 preparations, respectively.

2.3. *Uridine transport*

The transport of [^{3}H]uridine in plasma membrane vesicles was measured using a rapid filtration procedure described in [24]. All the specific characteristics of transport measurements related to the use of uridine as substrate have also been reported before [18]. In that previous report we showed that under our incubation conditions no significant metabolism of uridine is observed. The kinetic studies were carried out at 3 s (initial velocity conditions), in the presence of a transmembrane gradient of either NaSCN or KSCN (both at 100 mM) and substrate concentrations ranging from 0.25 to 50 μM. All experiments were performed at 22°C. Modifications of the incubation conditions needed for the monensin experiments will be given in section 3.

All transport experiments were carried out in triplicate in at least four different independent vesicle preparations. R_6 preparations were obtained after pooling two to three regenerating livers.

2.4. *Data analysis*

The analysis of sodium-dependent uridine uptake was carried out using the Enzfitter program (Elsevier, Biosoft, Cambridge). All data are expressed as mean ± S.E.M. Statistical comparisons between C_6 and R_6 samples were performed using the Student's *t*-test.

3. RESULTS

3.1. *Plasma membrane preparations*

The profile of the plasma membrane proteins of different preparations was studied and a representative result is shown in Fig. 1. The pattern shows high molecular weight proteins which suggests that the fractions did not undergo significant proteolysis. Furthermore, the profile was identical in C_6 and R_6 samples, thus suggesting that both preparations consisted of membranes of similar origin, showing roughly equal amounts of the major proteins. The immunoblot analysis (not shown) proved that these vesicles contained membranes from both domains, sinusoidal and canalicular. Thus, the finding of Na^+-dependent uridine uptake in our preparations is compatible with the subcellular localization of this carrier suggested by other groups [16,17].

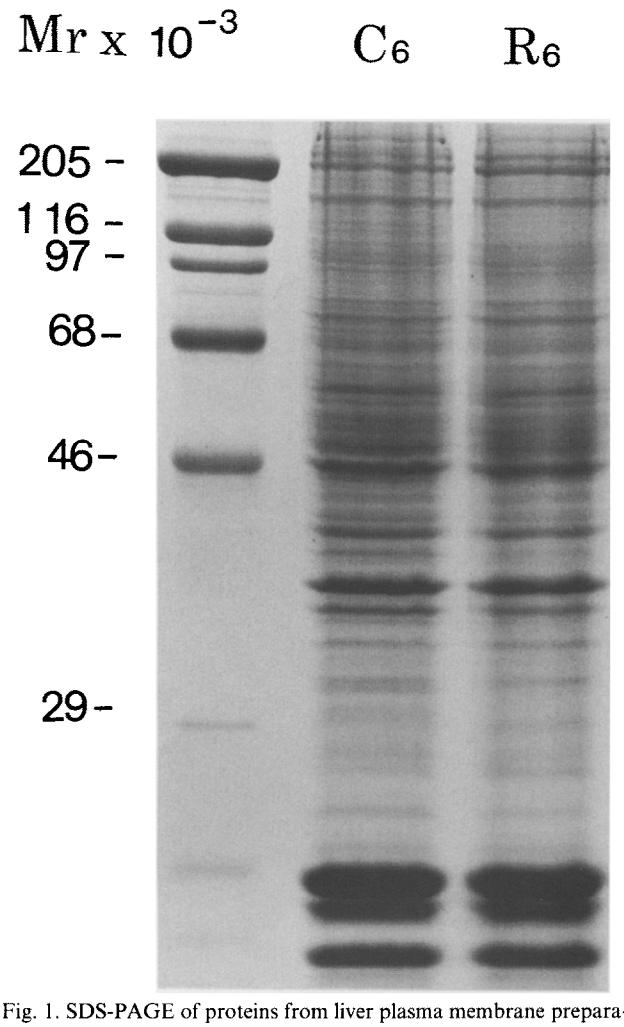

Fig. 1. SDS-PAGE of proteins from liver plasma membrane preparations. Membrane samples (100 μg protein) from either hepatectomized (R_6) or sham-operated (C_6) rats were run as indicated in section 2 and stained with Coomassie blue. The first lane corresponds to a mixture of proteins of known molecular weight.

3.2. *Kinetics of uridine uptake*

The effect of substrate concentration on Na^+-dependent uridine uptake by rat liver plasma membrane preparations was monitored and the results are shown in Fig. 2. Hepatectomy induced a marked increase in the V_{max} of uridine transport (3.7 ± 0.2 vs. 1.4 ± 0.1 pmol/

mg prot/3 s, for hepatectomized and sham-operated rats respectively, $P < 0.001$), while the K_m remained unchanged (6.0 ± 0.8 μM and 6.1 ± 0.9 μM for C_6 and R_6, respectively). The kinetic parameters of sham-operated animals closely resemble those from the C_0 group, which proves that surgery by itself does not cause any significant change in Na^+-dependent uridine uptake. When the non-saturable component of transport, the one measured in the presence of a transmembrane gradient of K^+ instead of Na^+, was kinetically analyzed, we found that the K_d for the plasma membrane preparations from hepatectomized rats was significantly higher than the one measured in those preparations from sham-operated animals (0.21 vs. 0.16 s^{-1}). This suggests that the basal permeability of the plasma membrane is increased 6 h after partial hepatectomy. This effect does not seem to be specific, because when L-alanine was used instead of uridine a similar change was found (not shown). These observations can probably be explained by changes in the lipid composition of liver plasma membranes following partial hepatectomy, which might affect membrane fluidity and simple diffusion of substrates.

3.3. *Effects of monensin on Na^+-dependent uridine uptake*

To determine whether the reported changes in uridine transport following partial hepatectomy can be fully or partially explained by a differential dissipation of the Na^+ gradient in both vesicle preparations (C_6 and R_6), we measured uridine uptake in conditions where the Na^+ transmembrane gradient was abolished. Thus, we incubated the vesicles either in the absence or the presence of 20 μM monensin at the same time that 5 μM uridine transport was monitored. Since monensin was diluted in DMSO, control experiments were performed after adding similar amounts of the solvent, which never exceeded a 2% of the final volume. The results obtained are shown in Table I. As expected, the addition of monensin to the NaSCN medium made nucleoside uptake rates decrease, however, no changes were observed when KSCN was used instead of NaSCN. Even in the presence of the ionophore, the difference between both experimental groups, R_6 and C_6, remained at a similar level, which suggests that the induction of the Na^+-dependent uridine transport occurring after partial hepatectomy does not rely upon a difference in the dissipation velocity of the transmembrane Na^+ gradient.

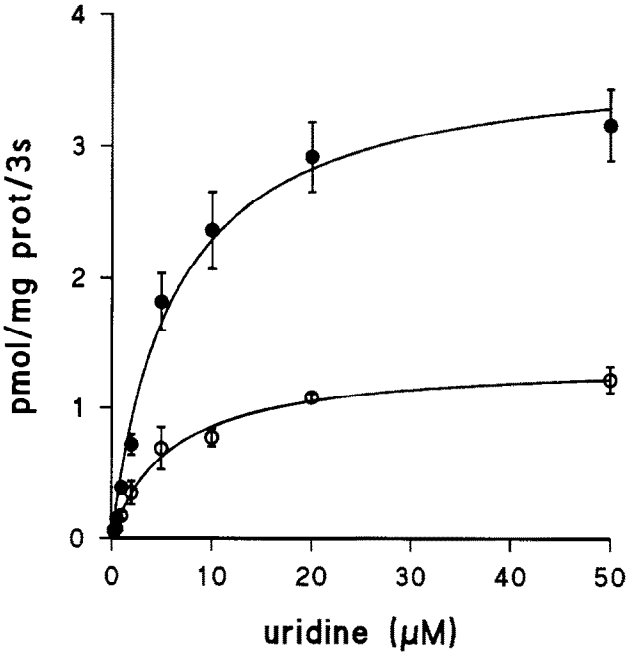

Fig. 2. Effect of substrate concentration on Na^+-dependent uridine uptake. Uridine uptake into plasma membrane vesicles from either 6-h regenerating (dark circles) or sham-operated (open circles) rats was monitored in the presence of increasing substrate concentrations (from 0.25 μM to 50 μM). Results are the mean ± S.E.M. of triplicate estimations made on four independent plasma membrane preparations.

4. DISCUSSION

Among the early events following partial hepatectomy in the rat and leading to liver cell proliferation, the hyperpolarization of the plasma membrane and the induction of Na^+-dependent amino acid transporters might play a key role as permissive factors helping the quiescent liver parenchymal cells getting into mitosis until full recovery of the liver mass (for review see [32]). Induction of Na,K-ATPase activity as well as subunit gene expression also occur early after partial hepatectomy [33]. These adaptations may be the consequence of the increasing demands of the cell for substrates of protein synthesis and largely precede the beginning of DNA synthesis occurring between 12 and 14 h after surgery.

The recent characterization of a Na^+-dependent nucleoside transporter in liver parenchymal cells [16–18]

Table I

Effect of monensin addition on uridine uptake. Liver plasma membrane vesicles from either 6-h regenerating (R_6) or sham-operated (C_6) rats were incubated with or without monensin (20 μM) and 5 μM uridine uptake was monitored either in a NaSCN or a KSCN medium. Results are the mean ± S.E.M. of nine estimations made on six independent pooled fractions.

	Uridine uptake (pmol/mg prot/3 s)	
	R_6	C_6
NaSCN medium	2.7 ± 0.16	1.4 ± 0.05
NaSCN medium + monensin	2.3 ± 0.10	1.2 ± 0.05
KSCN medium	0.9 ± 0.05	0.7 ± 0.04
KSCN medium + monensin	1.0 ± 0.03	0.7 ± 0.13
Na^+-dependent uptake	1.8	0.7
Na^+-dependent uptake + monensin	1.3	0.5

raises the question of whether the activity of this carrier can be somehow regulated. If the physiological role of this transport system is to preserve extracellular nucleosides for the endogenous synthesis of nucleic acids [17], then the activity of the carrier could be enhanced in response to a potent mitogenic stimulus like a partial hepatectomy. In this study we show that the V_{max} of uridine transport is 2.7-fold higher in those plasma membrane preparations from hepatectomized rats than in those preparations from sham-operated animals. This induction is observed as soon as 6 h after surgery and 6 to 8 h before the beginning of DNA synthesis occurs. Considering that this carrier shows broad specificity and could accept other natural nucleosides as substrates [18], the possibility that this adaptation confers the hepatocyte a greater disposal of substrates for nucleic acid synthesis should be taken into account. Interestingly, epithelial cells showing high turnover also possess Na^+-dependent nucleoside uptake [4,6,8–13], although, as reported above, the molecular identity of these transporters may be distinct [15]. Also HL-60 cells, when induced to differentiate, express a Na^+-dependent nucleoside transport system, of which it is not clear yet whether or not it corresponds to any of the agencies reported in other cell types [34]. In any case, it seems rather probable that a concentrative nucleoside carrier may underlie in the cellular mechanisms leading to cell proliferation. Assuming this is true, we speculate about the possibility that the activity of this transporter could be enhanced in other physiological situations leading to liver hypertrophia and hyperplasia.

Coming to the biochemical mechanism leading to this activation of uridine uptake, the experiments with monensin rule out the possibility that differences in the dissipation of the Na^+ gradient might be responsible for the enhancement of V_{max} found in the rat livers 6 h after partial hepatectomy. Thus, our data are compatible with an increase in the number of carriers, even more if we consider that no changes in the apparent K_m for the substrate were observed. Considering the hyperpolarization of the plasma membrane occurring early after hepatectomy and involving the transmembrane Na^+ gradient [33], we think that a first non-specific induction of most Na^+-dependent transporters could be triggered by protein-synthesis independent mechanisms linked to a transmembrane ionic unbalance, but, as a second early response, the specific induction of Na^+-dependent transporters, like the nucleoside one, would occur prior to the peak of DNA synthesis. The endocrine factors contributing to this enhancement remain to be established, but the possibility that this transport system could be a potential target of growth factor action seems especially attractive to us and is currently under investigation.

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