Hormonal regulation of concentrative nucleoside transport in liver parenchymal cells

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Na⁺-dependent uridine uptake is stimulated in isolated rat liver parenchymal cells by glucagon. This effect is transient, reaches maximum levels of stimulation 10 min after hormone addition, and is dose-dependent. Glucagon action can be mimicked by agents that are able to hyperpolarize the plasma membrane (e.g. monensin) and by dibutyryl cyclic AMP. The effects triggered by glucagon, monensin and dibutyryl cyclic AMP are not additive, suggesting a common mechanism of action. 8-(4-Chlorophenylthio)adenosine 3':5'-cyclic monophosphate (PCT), a cyclic AMP analogue but also a nucleoside analogue, markedly stimulates Na⁺-dependent uridine uptake in an additive manner to that triggered by monensin, similarly to the effect described for nitrobenzylthioinosine. Considering the roles reported for

nucleosides in liver metabolism, the use of PCT as a cyclic AMP analogue should be precluded. Insulin is also able to up-regulate Na⁺-dependent uridine uptake by a mechanism which involves a stable induction of this transport activity at the plasma-membrane level. This is consistent with a mechanism involving synthesis and insertion of more carriers into the plasma membrane. It is concluded that the recently characterized hepatic concentrative nucleoside transporter is under short-term hormonal regulation by glucagon, through mechanisms which involve membrane hyperpolarization, and under long-term control by insulin. This is the first report showing hormonal modulation of the hepatic concentrative nucleoside transporter.

INTRODUCTION

Nucleoside uptake by liver parenchymal cells is mediated by two types of transport activities: a concentrative Na⁺-dependent agency [1–3] and a nitrobenzylthioinosine (NBTI)-insensitive equilibrative transport component (J. Mercader, M. Gómez-Angelats, B. del Santo, J. Casado, A. Felipe and M. Pastor-Anglada, unpublished work). The Na⁺-dependent transport system, initially characterized in rat liver plasma-membrane vesicles, shows broad substrate specificity and appears to be electrogenic, with a likely stoichiometry of 1 nucleoside/1 Na⁺ [1–3]. The K_m values reported for its substrates are 6–12 μ M for uridine [2,4] and 8–14 μ M for adenosine [1,3]. These values are in the range of physiological concentrations of nucleosides [5]. Although concentrative nucleoside transporters are expressed only in a few specialized cell types, with little or no endogenous nucleoside-synthesis capacity (mainly absorptive epithelia), the physiological requirement for this agency in liver parenchymal cells is not so clear. Nevertheless, a bile-canalicular Na⁺/nucleoside co-transporter has been cloned very recently from rat liver [6], and it is evident that hepatocytes clearly concentrate nucleosides intracellularly, thus reaching values far higher than those found in blood [5]. Indeed, the homeostasis of these compounds may be of regulatory interest in liver parenchymal cells, where several key metabolic pathways are modulated by nucleosides (e.g. adenosine) through mechanisms involving their transport across the plasma membrane, rather than being receptor-mediated [7–9]. In most other cell types, transport and metabolism are tightly coupled in order to keep cytosolic concentrations low, thus facilitating nucleoside transport across the plasma membrane by equilibrative Na⁺-independent transport systems [10].

The evidence that concentrative nucleoside uptake is enhanced in the pre-replicative phase soon after partial hepatectomy [4] strongly suggests that this transport system is regulated in a complex way. In an attempt to elucidate the various hormones and growth factors able to modulate nucleoside transport in liver parenchymal cells, in the present study we analysed the regulation of Na⁺-coupled nucleoside transport in rat hepatocytes by glucagon and insulin. Although extensive information is available on the regulation of the plasma-membrane potential in liver parenchymal cells [11], there is little evidence that membrane potential regulates the performance of those plasma-membrane transporters accepting organic solutes as substrates. In this study we show that glucagon produces a transient stimulation of Na⁺-dependent uridine uptake in isolated rat hepatocytes. This effect was mimicked by dibutyryl cyclic AMP and other agents inducing membrane hyperpolarization. Glucagon action on Na⁺-dependent nucleoside transport may be of physiological relevance, according to the accepted role of this peptide in the early phases of liver regeneration after partial hepatectomy. Insulin is also able to up-regulate this transport activity by a stable mechanism. This is the first report showing hormonal modulation of the concentrative nucleoside transport activity of liver parenchymal cells.

MATERIALS AND METHODS

Isolation of hepatocytes

Hepatocytes were isolated from male Wistar rats (200–220 g body wt.) by an adaptation of the classical collagenase perfusion method, as previously described [12]. The final cell preparations were resuspended in a buffer containing 1.3 mM K₂HPO₄, 5.4 mM KCl, 0.44 mM KH₂PO₄, 0.98 mM MgCl₂ and either

Abbreviations used: PCT, 8-(4-chlorophenylthio)adenosine 3':5'-cyclic monophosphate; NBTI, nitrobenzylthioinosine.

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137 mM NaCl or 137 mM choline chloride. Mean cell viability was monitored by exclusion of 0.4% Trypan Blue and always exceeded 93%.

Transport measurements

Nucleoside uptake by isolated rat liver parenchymal cells was measured as previously described [13]. Cells resuspended in either NaCl or choline chloride, at a final concentration of $(0.5\text{--}1.5) \times 10^6$ cells/ml, were incubated for the indicated times in an equal volume of buffer, supplemented with [5,6- ^3H]uridine, at a final specific radioactivity of 2500 d.p.m./pmol. Uridine was used for transport measurements because it is poorly metabolized in isolated hepatocytes, as determined by chromatographic analysis of uridine-related metabolites from extracts of cells exposed to the radiolabelled substrate for different times, as previously described [2]. When the incubation time had elapsed, samples (0.25×10^6 cells) were collected and added to ice-cold Eppendorf tubes, containing an upper buffer phase, an intermediate oil layer [dibutyl phthalate/bis-(3,5,5-trimethylhexyl)-phthalate (3:1, v/v)] and a lower layer of 10% HClO_4 in glycerol. The tube was immediately centrifuged (14000 *g* for 60 s), the supernatants were aspirated and the pellet was counted for radioactivity. D-[1- ^{14}C]Mannitol was included in the incubation media to estimate the amount of extracellular medium trapped in the acid layer. Uptake rates were expressed as pmol of uridine/min per 10^6 cells. Na^+ -dependent transport was calculated by subtracting the uptake rates measures in choline chloride from those measured in NaCl. More details on the incubation conditions and treatments are given below in the explanation of the design of each particular experiment.

Measurement of membrane potential

Changes in membrane potential in isolated hepatocytes were monitored by measuring the ^{36}Cl in/out distribution ratio, as previously described by Bradford et al. [14]. Briefly, cell suspensions were incubated for 30 min in a medium identical with that used for transport experiments but containing Na^{36}Cl (4.5 $\mu\text{Ci}/\text{ml}$) and [^3H]inulin, the latter being used as a marker of trapped extracellular space. To monitor the effect of glucagon and other compounds on membrane potential, cells were incubated in the presence of these putative effectors for the last 10 min of incubation. This time point was chosen according to the time course of the glucagon effect on Na^+ -dependent nucleoside transport activity shown in Figure 2. Parallel experiments were performed to determine intracellular volume, by using the distribution space of $^3\text{H}_2\text{O}$, and [^{14}C]mannitol as extracellular marker. Incubations and separation of cells from the medium were performed as described in detail above for nucleoside transport measurements. For each determination, quadruplicate samples were taken and ^3H and ^{36}Cl radioactivities were determined by dual-channel liquid-scintillation counting. Radioactivity counts in cell pellets were then corrected on the basis of the trapped extracellular space. Cell membrane potential was calculated from the chloride distribution by using the Nernst equation.

Primary culture of liver parenchymal cells

Primary cultures of liver parenchymal cells were established immediately after hepatocyte isolation by seeding the hepatocytes on 35 mm Petri tissue-culture dishes at a mean density of 10^5 cells/ cm^2 . They were cultured for 4 h in 2%-fetal-calf-serum-supplemented 199 medium, already containing NaHCO_3 , glutamine and Earle's balanced salt solution. To allow efficient

attachment of cells to the plates, 200 nM dexamethasone was added during this period of time. Then the medium was replaced by a similar 199 medium, but with 0.2% BSA instead of serum. An appropriate mixture of antibiotics was also used to avoid contamination. Measurements of uridine transport into the hepatocytes were performed in the incubation media indicated above for the uptake determinations on suspensions of liver parenchymal cells. In this case, the need for an extracellular marker was unnecessary, because monolayers could be easily washed with cold stop buffer without significant loss of intracellular radioactivity and no extracellular contamination.

Euglycaemic/hyperinsulinaemic clamp, isolation of liver plasma-membrane vesicles and transport measurements

Male Wistar rats (200–220 g body wt.) were used. Rats were chronically catheterized under pentobarbital anaesthesia (60 mg/kg body wt., intraperitoneally) as previously described [15]. Experiments were always performed after full recovery from the surgical stress, determined on the basis of regularization of food intake. Fed and 24 h-fasted rats were infused with either saline (control group) or insulin (3 units/h per kg body wt.) (hyperinsulinaemic group). Hyperinsulinaemic animals were simultaneously infused with glucose at a variable rate (from 1.8 up to 3.9 g/h per kg body wt.) to maintain normoglycaemia. Glucose levels were routinely monitored by both a Glucometer (Bayer, Germany) and a rapid enzymic method, based on a coupled hexokinase/glucose-6-phosphate dehydrogenase assay (Boehringer, Mannheim, Germany), that allowed an accurate estimation of blood glucose in less than 2 min. Insulin was quantified at the end of the experiments in protamine-supplemented plasma with a commercial kit (CIS Bio International, Gif-sur-Yvette, France) and rat insulin as standard (Novo-Biolabs, Bagsvaerd, Denmark). After 6 h of either insulin or saline infusion, rats were killed by injection of an overdose of pentobarbital into one of the catheters. The liver was immediately excised and used for the isolation of plasma-membrane vesicles, as previously described [16]. This method has been extensively validated in our laboratory and involves a Percoll density gradient, which yields high recovery of plasma membrane, with selective enrichments of the basolateral domain. The preparations were characterized as previously reported [15,16]. Uptake measurements were performed by a rapid filtration method which has been extensively detailed and discussed previously [2,15–17]; 1 μM [^3H]uridine (sp. radioactivity 50 $\mu\text{Ci}/\mu\text{mol}$) was used as a substrate tracer.

RESULTS

Time course of uridine uptake by isolated hepatocytes

Uptake of 0.5 μM uridine by isolated rat hepatocytes was linear during the incubation time tested (2 min) in both NaCl and choline chloride media (Figure 1). Na^+ -dependent transport accounted for 50% of total uptake. All the experiments designed to explore hormone action were performed with 1 min as incubation time.

Effect of glucagon on Na^+ -dependent uridine uptake in isolated hepatocytes

Uridine uptake was measured in freshly isolated rat hepatocytes at the indicated times after 300 nM glucagon addition (Figure 2A). Glucagon exerted a rapid stimulation of Na^+ -dependent nucleoside transport into hepatocytes, which was significant 5 min after hormone addition and reached maximal values 10 min

Figure 1 Time course of uridine uptake into isolated rat liver parenchymal cells

Uptake of $0.5 \mu\text{M}$ uridine was monitored either in a NaCl (●) or in a choline chloride (○) medium. Na^+ -dependent uptake was calculated from these experimental data and is shown as ■. The results are means $\pm$ S.E.M. of triplicate measurements made on three independent cell preparations.

Figure 2 Effect of glucagon on Na^+ -dependent uridine uptake in isolated rat hepatocytes

(A) Glucagon (300 nM) was added to the hepatocytes, and transport measurements were performed at the indicated times, as explained in the Materials and methods section; $0.5 \mu\text{M}$ uridine was used as substrate. Results are expressed as the percentage hormonal induction above basal values (absence of glucagon). Basal values ranged from 1.7 to 4 pmol of uridine/min per 10^6 cells. Results are means $\pm$ S.E.M. of triplicate estimations made on three independent hepatocyte preparations. (B) Hepatocytes were incubated for 10 min in the presence of increasing concentrations of glucagon. At this time, $0.5 \mu\text{M}$ uridine uptake was monitored. Results are means $\pm$ S.E.M. of triplicate estimations made on two independent preparations.

after glucagon treatment. This effect was transitory and fully disappeared after 45 min of incubation. The effect was also dose-dependent (Figure 2B). A concentration of 10 nM glucagon was enough to induce stimulation of Na^+ -dependent uridine uptake, but the maximal effect was observed at 100 nM . Maximal induction was in the range of 80 – 130% above basal values (absence of hormone), depending on the hepatocyte preparation used. Glucagon did not modify the Na^+ -independent uridine uptake rates when measured in a choline chloride medium

Figure 3 Effect of monensin on Na^+ -dependent uridine uptake in isolated rat hepatocytes

Hepatocytes were incubated for 10 min in the presence of increasing concentrations of monensin. Thereafter, $0.5 \mu\text{M}$ uridine uptake was determined. Results are means $\pm$ S.E.M. of triplicate estimations made on three independent preparations.

Table 1 Effect of glucagon, monensin and dibutylrly cyclic AMP on Na^+ -dependent uridine uptake and membrane potential in isolated rat liver parenchymal cells

Uptake of $0.5 \mu\text{M}$ uridine and membrane-potential measurements were performed as indicated in the text. The effectors were added at the following concentrations: 300 nM glucagon, $30 \mu\text{M}$ monensin, 1 mM dibutylrly cyclic AMP. Relative induction is shown in parentheses as percentage of the basal values (cells incubated without effectors). Results are means $\pm$ S.E.M. of triplicate estimations made on four independent cell preparations. Statistical significance was assessed by the paired t test (* $P < 0.05$; ** $P < 0.01$).

	Na^+ -dependent uridine uptake (pmol of uridine/min per 10^5 cells)	Membrane potential (mV)
No additions	1.8 ± 0.3	-35.8 ± 1.5
Glucagon	$3.7 \pm 0.3^{**}$ (105%)	$-43.0 \pm 2.2^*$ (20%)
Monensin	$3.2 \pm 0.4^*$ (78%)	$-43.0 \pm 3.1^*$ (20%)
Dibutylrly cyclic AMP	$3.3 \pm 0.5^*$ (83%)	$-45.4 \pm 3.2^*$ (27%)
Glucagon + monensin	$3.5 \pm 0.3^{**}$ (94%)	$-43.5 \pm 4.7^*$ (21%)
Monensin + dibutylrly cyclic AMP	$3.6 \pm 0.4^*$ (100%)	$-47.9 \pm 3.4^*$ (33%)

(results not shown). The effect triggered by glucagon was mimicked by the addition of a Na^+/H^+ ionophore, monensin (Figure 3), known to induce hyperpolarization of the plasma membrane (Table 1). This effect was also dose-dependent, inducing a maximal stimulation of 90% above control values. Dibutylrly cyclic AMP also enhanced Na^+ -dependent uridine transport in freshly isolated rat hepatocytes. This effect was not additive to that triggered by monensin (Table 1), nor was the effect induced by monensin and glucagon when added together (Table 1). In all the conditions tested, those agents able to up-regulate Na^+ -dependent nucleoside transport into liver parenchymal cells also induced plasma-membrane hyperpolarization (Table 1).

Table 2 Effect of the nucleoside analogues PCT and NBTI on Na⁺-dependent uridine uptake in isolated rat liver parenchymal cells

Uptake of 0.5 μ M uridine was determined as indicated in the text. The effectors were added at the following concentrations: 30 μ M monensin, 1 mM PCT, 10 nM NBTI. Relative induction is shown in parentheses as percentage of the basal values (cells incubated without effectors). Results are means $\pm$ S.E.M. of triplicate estimations made on four independent cell preparations. Statistical significance was assessed by the paired *t* test (* *P* < 0.05; *** *P* < 0.001).

	Na ⁺ -dependent uridine uptake (pmol of uridine/min per 10 ⁶ cells)
Basal	1.3 $\pm$ 0.1
Monensin	2.5 $\pm$ 0.5* (92%)
PCT	7.0 $\pm$ 0.7*** (430%)
Monensin + PCT	8.6 $\pm$ 0.6*** (560%)
NBTI	4.8 $\pm$ 0.2*** (270%)
Monensin + NBTI	6.5 $\pm$ 0.3*** (399%)

Figure 4 Na⁺-dependent uridine uptake in freshly isolated rat hepatocytes and primary cultures of liver parenchymal cells

Freshly isolated hepatocytes were kept in suspension for 3 h, and rates of uptake of 1 μ M uridine were measured at the indicated time points (○). In parallel, cells were seeded on tissue-culture dishes, as indicated in the text, and 1 μ M uridine uptake was determined after 4 h of plating (the minimum time to ensure adhesion of cells to the plates). Then uptake rates (pmol/min per mg of protein) were monitored at the indicated times (●) until 3 days after plating. Results are means $\pm$ S.E.M. of triplicate estimations made on four independent cell preparations.

Interestingly, another common membrane-permeant analogue of cyclic AMP, 8-(4-chlorophenylthio)adenosine 3':5'-cyclic monophosphate (PCT), strongly stimulated Na⁺-dependent uridine uptake in liver parenchymal cells (up to 4-fold above basal values) (Table 2). A similar effect in terms of magnitude was observed when using NBTI (nearly 3-fold above basal values), a nucleoside analogue known to inhibit Na⁺-independent nucleoside uptake in most cell types. Indeed, PCT and NBTI effects on Na⁺-dependent uridine uptake were clearly additive to those triggered by monensin, thus supporting the view that these analogues are up-regulating this transport activity by a different mechanism from that induced by the ionophore. Indeed, PCT did not significantly modify membrane potential in these conditions (results not shown). Consistent with their structural similarities, the stimulatory effect of PCT may be mediated by the same mechanism as NBTI. Neither PCT nor NBTI sig-

Table 3 Effect of insulin on Na⁺-dependent uridine uptake

Expt. I: 24 h primary cultures of rat hepatocytes were exposed to 100 nM insulin for 3 h. Then 1 μ M uridine uptake was measured in either a NaCl or a choline chloride medium. Results are means $\pm$ S.E.M. of triplicate estimations made on ten independent cell cultures. Statistical differences between basal and insulin-stimulated uptake rates were established by a paired *t* test (* *P* < 0.05; ** *P* < 0.01). Expt. II: plasma-membrane vesicles were partially purified from rats that had been kept for 6 h under a euglycaemic/hyperinsulinaemic clamp, as indicated in the text. Saline-infused rats were used as controls (Basal conditions). Then 1 μ M uridine uptake was measured in either a NaSCN or a KSCN medium. Results are means $\pm$ S.E.M. of triplicate estimations made on five independent preparations from either hyperinsulinaemic or normoinsulinaemic rats. Statistical significance of the effect induced by insulin was assessed by Student's *t* test (** *P* < 0.01; *** *P* < 0.001). The difference between basal uptake rates in those preparations from fed and fasted rats was also statistically significant (††† *P* < 0.001).

		Uptake (pmol of uridine/3 min per 10 ⁶ cells)	
		Basal	Insulin
Expt. I Primary cultures			
NaCl medium		0.56 $\pm$ 0.03	0.72 $\pm$ 0.06*
Choline chloride medium		0.36 $\pm$ 0.03	0.39 $\pm$ 0.03
Na ⁺ -dependent transport		0.20 $\pm$ 0.05	0.33 $\pm$ 0.05*
		Na ⁺ -dependent transport (pmol of uridine/3 s per g of protein)	
		Basal	Hyperinsulinaemic
Expt. II Plasma-membrane vesicles			
Fed rats	No ionophore	153 $\pm$ 5	228 $\pm$ 5***
	+ 25 μ M Monensin	25 $\pm$ 5	48 $\pm$ 5**
Fasted rats	No ionophore	102 $\pm$ 9†††	232 $\pm$ 15***

nificantly modified uridine uptake rates when measured in a choline chloride medium (results not shown).

Na⁺-dependent uridine uptake in primary cultures of rat hepatocytes and the insulin effect

Na⁺-dependent uridine uptake rates in isolated hepatocytes and in primary cultures of these cells are shown in Figure 4. Transport activity remained relatively constant in suspensions of rat hepatocytes for at least 3 h (the longest time examined), but markedly decreased when cells were seeded for primary culture. Basal Na⁺-dependent transport activity was depleted to less than 10% of the original activity in cell suspensions when uptake rates were measured at the first time point monitored in this study (4 h of culture). This low transport rate was kept more or less constant for the first 24 h, and significantly increased (around 2-fold) after 3 days of culture. In these conditions, transport rates were still much lower than those found in suspensions of isolated liver parenchymal cells. The effect of insulin on Na⁺-dependent uridine uptake was monitored 24 h after plating the cells; 100 nM insulin significantly and selectively increased the Na⁺-dependent component of uridine transport (Table 3). However, the low uptake rates found in primary cultures precluded a more detailed study of this effect. Thus, we monitored the likely effects of the hormone using an approach *in vivo* previously used in our laboratory, the euglycaemic/hyperinsulinaemic clamp [15]. Insulin levels after 6 h of hormone infusion reached nearly 5000 μ -units/ml in both fed and 24 h-fasted rats. This was achieved without perturbing the glycaemia, which was kept constant during the experiment. Insulin levels of saline-infused fed and fasted rats were 90 $\pm$ 10 and 42 $\pm$ 5 μ -units/ml respectively. In both fed and fasted states, insulin was able to up-regulate the

Na⁺-dependent uridine transport activity, as deduced from the evidence that uptake rates were higher in those plasma-membrane vesicle preparations from hyperinsulinaemic rats (Table 3). Interestingly, the effect was more pronounced in relative terms in fasted animals. This is due to the fact that starvation by itself significantly decreases basal Na⁺-dependent uridine transport activity. In all these conditions, uridine uptake, when measured in a KSCN medium, was not significantly affected. As an attempt to determine whether this induction could be related to different dissipation rates of the Na⁺ electrochemical transmembrane gradient, we measured Na⁺-dependent uridine uptake in vesicles which had been treated with 25 μ M monensin, a Na⁺ ionophore. Under these conditions, uptake rates were much lower than in the absence of monensin, but the differences between groups, saline-infused and hyperinsulinaemic rats, remained.

DISCUSSION

This study shows, for the first time, that concentrative nucleoside transport activity expressed in liver parenchymal cells is under hormonal regulation by short-term mechanisms, which may involve membrane hyperpolarization, and by long-term mechanisms, which are consistent with synthesis *de novo* of carrier proteins.

The membrane potential of hepatocytes is kept within a relatively narrow range, reported to be around $-25/-35$ mV, depending on the experimental model and the technique used for measurements [11,18]. This is consistent with the hepatocyte being a non-excitable cell type. Nevertheless, membrane potential can be experimentally modified in a wider range ($-20/-60$ mV), either by treatment with hormones (e.g. glucagon) or by various drugs, such as ouabain and ionophores [11,18,19]. A close relationship has been shown between Na⁺-dependent alanine uptake and membrane potential [11,18,20]. It has long been known that the perturbation of the Na⁺ gradient affects alanine transport, and even interferes with the determination of the coupling ratio between both co-substrates [11,19–25]. This is in accordance with the electrogenic properties of the amino acid transport systems involved in alanine transport into liver parenchymal cells, systems A and ASC. System A rather than ASC activity has been shown to have a linear correlation with the Na⁺ electrochemical potential [25]. In accordance with this finding, evidence for short-term regulation of concentrative neutral amino acid transport into liver parenchymal cells has been provided [26]. Glucagon and epidermal growth factor induce a transient stimulation of Na⁺-dependent L-alanine uptake in hepatocytes by a mechanism mimicked by nigericin, a Na⁺/H⁺ ionophore known to hyperpolarize the plasma membrane [19,26]. The effects triggered by dibutyryl cyclic AMP and nigericin are not additive. Neither were those induced by dibutyryl cyclic AMP and monensin on Na⁺-dependent uridine uptake, reported in the present work. The time course of the effect, as well as its magnitude, is similar for both types of substrate, amino acids and nucleosides. This finding is consistent with the coupling ratio of the nucleoside transporter and its suggested electrogenicity. Thus Na⁺-linked nucleoside uptake is dependent on membrane potential in isolated hepatocytes, and consequently it is sensitive to those agents able to modify this parameter.

The effect of glucagon on nucleoside transport may have physiological relevance. Portal glucagon levels increase soon after partial hepatectomy in the pre-replicative phase of liver growth [27]. A transient stimulation of amino acid and nucleoside uptake triggered by this pancreatic hormone would provide key substrates for the early steps of liver-cell proliferation. Nevertheless, considering that the effect is transitory, it is unlikely that

this accounts for the stable change in Na⁺-dependent uridine uptake found in liver plasma-membrane vesicles from hepatectomized rats. Hepatectomy resulted in a $V_{\max}$ effect, which was stable and independent of the Na⁺ electrochemical gradient [4]. Thus, other hormones and/or growth factors may further up-regulate this transport system by a mechanism involving synthesis *de novo* of carrier proteins.

In this context, this study also shows that insulin enhances Na⁺-dependent uridine uptake, both in primary cultures of rat hepatocytes and in plasma-membrane vesicles from euglycaemic/hyperinsulinaemic rats. Primary cultures of rat hepatocytes show a marked decrease in uridine uptake rates, which, in some cases, results in almost negligible Na⁺-dependent activity. This further supports the view that this transport system may have complex trophic requirements, but complicates its study in primary cultures of rat hepatocytes. We are now determining the requirements for this transport activity to be maintained in cultured cells.

The clamp technique has been used recently in our laboratory to explore the insulin-sensitivity of system A for neutral amino acid transport *in vivo* [15]. This method is based on the possibility of inducing hyperinsulinaemia in animals under normoglycaemic conditions, and it has been widely used to study glucose transport [28]. Indeed, the effect found *in vitro* by using physiological doses of insulin is reproduced *in vivo* with the clamp technique, although this time we only used supramaximal doses of the hormone. Nevertheless, the requirement for insulin of the Na⁺-dependent uridine transport activity is further supported by the indirect evidence that starvation by itself induces a decrease in this parameter. Indeed, fasting is characterized by insulin deficiency. The evidence that treatment of the vesicles with monensin does not modify insulin action in relative terms is consistent with the idea that the hormone is inducing stable effects which may involve synthesis *de novo* of carrier proteins.

Considering that nucleosides, adenosine in particular, are known to modulate some key metabolic pathways in liver parenchymal cells, our results suggest that PCT should not be used when exploring the metabolic effects of glucagon in hepatocytes. This is a collateral but important finding of this contribution. The mechanisms involved in the induction of Na⁺-dependent nucleoside transport triggered by PCT and NBTI require further characterization. Nevertheless, the NBTI effect cannot be due to inhibition of passive efflux, because equilibrative transport in liver parenchymal cells is NBTI-insensitive. Other workers have reported an abundance of high-affinity NBTI-binding sites in liver membranes [29], but it does not necessarily mean that the NBTI-sensitive carriers are active. In this context, preliminary studies from our laboratory show that fetal hepatocytes express a Na⁺-independent nucleoside transport activity which is sensitive to NBTI, but is almost negligible in hepatocytes from newborns. This means that NBTI-sensitive transport activity may be developmentally regulated in liver parenchymal cells.

In summary, this contribution provides the first evidence for the hormonal regulation of hepatic concentrative nucleoside transport. The recent cloning of a putative nucleoside transporter from liver cells [6] will probably contribute to a better understanding of the ultimate molecular basis involved in the hormonal effects reported in this study.

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