Hyperosmolarity leads to an increase in derepressed system A activity in the renal epithelial cell line NBL-1

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Hyperosmolarity induced an increase in Na⁺-dependent L-alanine uptake in confluent monolayers of the established renal epithelial cell line NBL-1. This induction was attributable to system A and was only seen when the cells had been previously deprived of amino acids in the culture medium to derepress system A activity. It was additive to the adaptive regulation induction, and both were inhibited by cycloheximide. However, the hyperosmolarity effect was inhibited by colcemid (an inhibitor of microtubular function), but adaptive regulation was not. Otherwise, when cell monolayers were incubated in a con-

trol medium, basal Na⁺-dependent L-alanine uptake mediated by system B⁰ decreased. The results of this study show that: (i) system A activity was not induced by cell shrinkage and subsequent swelling due to extracellular hyperosmolarity when cells were incubated in control medium; (ii) previous expression of system A activity induced by amino acid starvation seems to be a prerequisite for further induction due to hyperosmolarity; and (iii) the effects of adaptive regulation and hyperosmotic stress are mediated by different mechanisms.

INTRODUCTION

Many neutral amino acids are taken up into mammalian cells by a transport system, not yet identified at the molecular level, called system A. This system, in addition to its natural substrates, shows specificity for *N*-methylated substrates, and its activity can be easily assessed either by measuring the uptake of *N*-methylaminoisobutyric acid (MeAIB) or the fraction of Na⁺-dependent neutral amino acid uptake inhibited by an excess of MeAIB. System A activity is regulated by nutritional and hormonal factors in a complex way, involving short- and long-term mechanisms of control. Among the latter, derepression of the gene coding for the system-A protein(s), as a consequence of amino acid starvation, is a common feature in all the cell types so far studied. Basically this effect consists of the protein-synthesis-dependent induction of system A activity, triggered by depriving the culture medium of amino acids (for reviews see Guidotti et al., 1978; Shotwell et al., 1983; Christensen, 1990). This response is regulated *in vitro*, because selected amino acids can inhibit the derepression of the gene. This feature gave rise to models of control of system-A gene expression in human fibroblasts, rat hepatocytes and the cultured cell line CHO-K1 (Gazzola et al., 1981; Shotwell et al., 1983; Moffett and Englesberg, 1984; Qian et al., 1991).

Established cell lines of renal origin have been widely used to study mechanisms of amino acid transport. However, there is no clear evidence for the presence of system A in these cells. Although the presence of system A has been reported in non-confluent MDCK cells (Boerner and Saier, 1985), in LLC-PK1 and opossum kidney (OK) cell lines no system A activity was found (Sepúlveda and Pearson, 1982; Schwegler et al., 1989). Recently, we have characterized the main system responsible for neutral amino acid uptake in the cultured renal epithelial cell line NBL-1, which can be identified as system B⁰ (Doyle and McGivan, 1992). This system is Na⁺-dependent, shows a broad specificity and closely resembles the agency characterized in

plasma-membrane vesicles from bovine renal cortex (Lynch and McGivan, 1987). Interestingly, this established cell line has proved to be an excellent model to study the regulation of system A expression. Although no system A activity is detectable when the cells are grown in a regular medium, it is induced when cells are cultured in an amino-acid-deprived medium for up to 6 h (Felipe et al., 1992). In accordance with the suggested models of system-A gene derepression, this response is inhibited by cycloheximide and is blocked by tunicamycin and also by selected amino acids, in a pattern which closely resembles that described in other cell types (Guidotti et al., 1978; Barber et al., 1983; Kilberg et al., 1985). Furthermore, this induction is accompanied by the incorporation of labelled mannose in a 113–140 kDa glycoprotein, which is in accordance with the suggested molecular mass of the system-A protein deduced by using other experimental approaches (McCormick and Johnstone, 1988; McCormick et al., 1991).

In the present study we further investigate the regulation of system A by examining the effect of increasing osmolarity on Na⁺-dependent L-alanine transport in confluent NBL-1 cell monolayers. So far, some metabolic functions of liver parenchymal cells, such as proteolysis, glycogen synthesis and system N activity, seem to be under short-term regulatory mechanisms involving changes in cell volume, essentially cell swelling (Baquet et al., 1990; Bode and Kilberg, 1991; Haüssinger et al., 1991; for review, see Haüssinger and Lang, 1991a). Indeed, liver parenchymal cell volume itself is supposed to be under hormonal control (Haüssinger and Lang, 1991a; vom Dahl et al., 1991b), and similar effects have been reported when studying amino acid release in isolated rat muscles (Parry-Billings et al., 1990). Long-term effects triggered by anisotonic media, requiring either protein synthesis or gene transcription or both, have been also reported in other cell types (García-Pérez et al., 1989; Poulin and Pegg, 1990). Some of the induced activities are related to the biochemical pathways leading to the synthesis of organic osmolytes. This feature may be especially important in kidney

Abbreviation used: MeAIB, 2-methylaminoisobutyric acid.

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epithelial cells (García-Pérez et al., 1989; Haüssinger and Lang, 1991b), although the effects of changes in cell volume on renal metabolic functions have been very little studied. In this work we show that system A activity is induced when confluent NBL-1 cell monolayers are cultured in hypertonic conditions, but only when system A has been previously expressed by amino acid starvation. The induction is additive, sensitive to cycloheximide and, in contrast with what is found for the derepression effect, it is blocked by the colchicine analogue colcemid.

EXPERIMENTAL

Materials

L-[³H]Alanine, [U-¹⁴C]urea and [³H]inulin were purchased from Amersham International (Amersham, Bucks., U.K.). Ham's F-12 medium, fetal-calf serum, antibiotic/antimycotic (10000 units of penicillin G/ml, 10 mg/ml streptomycin and 25 µg/ml amphotericin B), the amino acid analogue MeAIB, cycloheximide and colcemid were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Gentamycin (50 mg/ml) and L-glutamine (200 mM) were from Boehringer Mannheim (Germany). All other chemicals and reagents were of analytical grade.

Cell culture

NBL-1 cells were obtained from Flow Laboratories (McLean, VA, U.S.A.) and cultured in Ham's F-12 medium supplemented with 10% fetal-calf serum, 2 mM L-glutamine, 100 units/ml penicillin G, 0.1 mg/ml streptomycin, 0.25 µg/ml amphotericin B, 50 µg/ml gentamycin, at 37 °C in a gas phase of CO₂/air (1:19). Cells were grown in 75 cm² flasks for continuous culture and in 35 mm-diameter Petri dishes for experiments. Cells were fed every 2 days until confluence, when they were used for induction studies.

Derepression studies

To induce system A activity, confluent cells were incubated for the indicated times in a medium deprived of amino acids. Basically, the amino-acid-free medium consisted of the inorganic salt components of Ham's F-12 medium plus 10 mM glucose and 0.5% Phenol Red, supplemented with 0.1% BSA and antibiotics, as indicated above. These conditions have been previously shown to induce system A activity in NBL-1 cells (Felipe et al., 1992).

Hypertonic media

To increase the osmolarity of the culture media, either the control or the starvation media were supplemented with increasing concentrations of sucrose (50–300 mM). In some experiments hypertonicity was achieved by adding mannitol instead of sucrose. Both isotonic and hypertonic media, when indicated, were supplemented with inhibitors of protein synthesis (cycloheximide) and microtubular function (colcemid) (Diez et al., 1987). More details about the conditions are given in the Results section.

Transport measurements

Uptake of 0.1 mM L-[³H]alanine was measured in either the absence or the presence of 5 mM MeAIB, by a modification of the method described by Quamme et al. (1989), as previously reported (Doyle and McGivan, 1992; Felipe et al., 1992). Basically, cells were washed in the following medium:

137 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 1.2 mM MgSO₄, 10 mM Hepes, adjusted to pH 7.4 with Tris. To inhibit alanine metabolism, 0.5 mM amino-oxyacetate was added. Amino acid uptake was started by adding 1 ml of the above medium containing labelled substrate (1 µCi/ml). Na⁺-independent uptake of L-alanine was monitored by using 137 mM choline chloride instead of NaCl in the transport medium. The incubation was performed at 22 °C and stopped after 3 min by removing the transport medium and washing with 3 × 2 ml of an ice-cold solution of 137 mM NaCl/10 mM Tris/Hepes, pH 7.4. This time had been shown to be suitable for measurement of initial velocity (Doyle and McGivan, 1992; Felipe et al., 1992). Plates were drained and 0.5 ml of 0.5% Triton X-100 was added. Cells were then scraped off and dissolved. A 200 µl portion of this solution was added to 4 ml of scintillation fluid and counted for radioactivity. In preliminary experiments, transport medium was supplemented with 200 mM sucrose, to ascertain whether the occurrence of hypertonicity during the uptake measurements could modify basal rates. No differences were found when either incubation medium was used, and all the experiments were later performed without sucrose, as indicated above.

Protein content of individual plates was determined as previously described (Bradford, 1976). Then, all uptake rates were expressed as nmol of L-alanine/3 min per mg of protein.

Cell volume determination

To determine cell volume, plates were directly supplemented with [³H]inulin and [U-¹⁴C]urea to give a final radioactivity of 1 µCi/ml and 0.2 µCi/ml respectively. In a preliminary set of experiments, the time course of urea uptake was monitored. At different times, cells were immediately washed and scraped off, and samples were counted for radioactivity (³H/¹⁴C), as indicated above. To calculate intracellular urea content, ¹⁴C counts were corrected for contamination by extracellular medium (³H counts). Equilibrium was reached after 30 min. For all subsequent experiments, the markers were added 60 min before changing the media, which were also supplemented with labelled markers at equal specific radioactivity. Cells were processed as above, at the times indicated in the Results section.

RESULTS

Induction of Na⁺-dependent L-alanine uptake: effect of sucrose

In a first set of experiments we reproduced the findings of our previous study (Felipe et al., 1992), showing that NBL-1 cells express system A activity *de novo* when incubated in the absence of amino acids (Figure 1b). A 12 h incubation was enough to induce Na⁺-dependent L-alanine uptake significantly, as a consequence of system A derepression (Felipe et al., 1992; Table 1). As previously reported, the addition of 10 µg/ml cycloheximide prevented the induction (Felipe et al., 1992). No effects of cycloheximide by itself on the basal transport rates were observed (Figure 1a). When the starvation medium was supplemented with 200 mM sucrose from the beginning of the experiment, an induction of the Na⁺-dependent L-alanine transport, which was double that found in the amino-acid-free medium, was observed (Figure 1b). Addition of sucrose to cells cultured in control media did not result in any significant induction of the Na⁺-dependent L-alanine uptake, but rather it made basal transport rates decrease, which was already significant at 12 h. When cycloheximide was added to the amino-acid-free media from the beginning, no induction by sucrose was observed, and the changes paralleled those found in control conditions (Figure 1). When the experiments were performed with mannitol instead of sucrose,

Figure 1 Effect of sucrose on Na⁺-dependent L-alanine uptake in confluent monolayers of NBL-1 cells

NBL-1 cells were incubated for 24 h in the presence (a) or in the absence (b) of amino acids, with or without 200 mM sucrose. Where indicated, 10 μ g/ml cycloheximide (CHX) was added at the beginning of the experiment. At desired times, uptake was measured as described in the Experimental section. Symbols: (a) ●, control medium; ■, control medium + CHX; ○, control + sucrose; □, control + sucrose + CHX; (b) ●, amino-acid-free medium; ■, amino-acid-free medium + CHX; ○, amino-acid-free medium + sucrose; □, amino-acid-free medium + sucrose + CHX. Values are means $\pm$ S.E.M. of determinations on at least 3 different cell monolayers.

Figure 2 Dose-dependence of the effects of sucrose on Na⁺-dependent L-alanine uptake in NBL-1 cells

Confluent cell monolayers were exposed to different sucrose concentrations (0–300 mM) for 24 h. Alanine transport was measured as indicated in the Experimental section. (a) Na⁺-dependent L-alanine transport in both control and starved cells; (b) difference in Na⁺-dependent L-alanine transport between control and amino-acid-starved NBL-1 cells. Symbols: ●, control; ○, starved; ■, differences between groups. Values are means $\pm$ S.E.M. of 3–5 different experiments.

similar results were obtained (results not shown), thus showing that the effect was not specific to sucrose, but rather related to the hypertonicity of the media.

Dose-dependence of the effects induced by sucrose

The induction of the Na⁺-dependent L-alanine uptake by sucrose is dose-dependent. When cells were incubated for 24 h in the presence of increasing concentrations of sucrose, it was observed that, in the absence of amino acids, sucrose was able to enhance Na⁺-dependent L-alanine uptake further (Figures 2a and 2b). In a normal culture medium, sucrose made basal rates decrease (Figure 2a). When the latter uptake rates (control + sucrose) were subtracted from the induced rates (amino acid-free + sucrose), a dose-dependent curve of the effects of sucrose on derepressed Na⁺-dependent L-alanine uptake was obtained (Figure 2b). Sucrose concentrations above 100 mM exerted a significant induction. A maximal effect was already achieved at

200 mM, and further experiments were always performed with this concentration.

Sucrose addition enhances system A activity in a protein-synthesis-dependent manner

Although the derepression of system A had been shown to be a prerequisite for the induction of Na⁺-dependent L-alanine uptake triggered by sucrose, it had still to be determined whether this further induction could be attributed to an increase in this system activity. To dissect these two effects, a set of experiments was designed in which cells were incubated for 12 h in either the presence (repressed conditions) or the absence (derepressed conditions) of amino acids. Once the cells were derepressed, a second incubation for 12 h was performed in the presence of either 200 mM sucrose or 10 μ g/ml cycloheximide, or both. Those cells that had not been derepressed were also incubated for a further 12 h in the presence of sucrose. In all these conditions,

Table 1 Effect of sucrose on Na⁺-dependent alanine transport in confluent monolayers of NBL-1 cells

Cell monolayers were grown and processed as described in the Experimental section. After the first 12 h of incubation either in a control or in an amino-acid-free medium, cells were incubated for a further 12 h in a medium with or without amino acids, supplemented or not with sucrose (200 mM). Where indicated, 10 µg/ml cycloheximide (CHX) was added. (a) No second incubation was performed. Results are expressed as a percentage of the basal values (when cells were incubated in a control medium). Basal uptake rates were 2.37 ± 0.11 (9) nmol/3 min per mg of protein. Values are means $\pm$ S.E.M. of the numbers of determinations indicated in parentheses. Statistical comparisons (Student's *t* test) are: *** $P < 0.001$ versus no inhibitor; †† $P < 0.01$, ††† $P < 0.001$ versus basal.

Incubation conditions		Uptake (% of basal)	
0–12 h	12–24 h	No inhibitor	Inhibitor (5 mM MeAIB)
Control	—(a)	100.0 $\pm$ 5.0 (9)	96.8 $\pm$ 2.9 (4)
	Control + sucrose	73.0 $\pm$ 3.7 (5)†††	67.7 $\pm$ 1.3 (3)†††
Amino-acid-free	—(a)	153.5 $\pm$ 4.9 (6)†††	104.6 $\pm$ 6.6 (5)***
	Amino-acid-free	173.6 $\pm$ 3.9 (8)†††	99.3 $\pm$ 5.7 (4)***
	Amino-acid-free + CHX	165.0 $\pm$ 3.7 (4)†††	106.3 $\pm$ 3.4 (3)***
	Amino-acid-free + sucrose	231.5 $\pm$ 18.0 (4)†††	82.0 $\pm$ 1.0 (3)***††
	Amino-acid-free + sucrose + CHX	94.8 $\pm$ 4.4 (5)	53.0 $\pm$ 2.5 (3)***†††

uptake of 0.1 mM L-alanine was monitored in either the presence or the absence of 5 mM MeAIB. Results are shown in Table 1. It is shown, as previously reported (Felipe et al., 1992), that during the first 12 h of incubation of the cells in an amino-acid-free medium the induction of Na⁺-dependent L-alanine uptake was accounted for by system A and blocked by cycloheximide. When the cells were kept for a further 12 h in the amino-acid-deprived medium, system A activity further increased, although to a much lesser extent. However, when 200 mM sucrose was added during this second 12 h incubation period, a large enhancement in system A activity was observed, as deduced from the observation that the overall increase in Na⁺-dependent L-alanine uptake was inhibited by MeAIB. The induction of system A mediated by sucrose addition was also blocked by cycloheximide. However, in these conditions, the uptake rates fell to those of control repressed conditions.

Sucrose itself causes a significant decrease in basal Na⁺-dependent L-alanine uptake. A 12 h incubation of repressed cells with 200 mM sucrose resulted in a significant decrease in system B⁰ activity, as deduced from the finding that MeAIB was unable to inhibit L-alanine transport. However, when derepressed cells were incubated for a further 12 h in an amino-acid-free medium supplemented with sucrose and cycloheximide, system A activity was detected at a similar extent to that found in derepressed cells. In these conditions, only system B⁰ seemed to be affected by the sucrose treatment, as also observed in the control experiments (Figures 1 and 2, Table 1). In conclusion, induction of system A by both amino acid starvation and sucrose treatment requires protein synthesis and seems to be additive, thus suggesting that different mechanisms might be mediating the two actions.

Effect of colcemid on system A induction

To determine whether different mechanisms are involved in system A induction triggered by either amino acid starvation or

Table 2 Effect of 10 µg/ml cycloheximide or colcemid on Na⁺-dependent L-alanine transport in confluent monolayers of NBL-1 cells

In the first two sets of conditions cells were incubated for 12 h either in the presence or in the absence of cycloheximide (CHX) or colcemid (CLD). Control cells were incubated in a standard medium (Ham's F-12); 'Starved' cells were incubated for 12 h in an amino-acid-free medium. In the 'Starved + sucrose' condition (third set of experiments), the monolayers were incubated for 12 h in an amino-acid-free medium and for a further 12 h in the same medium supplemented with 200 mM sucrose with or without CHX or CLD. See the Experimental section for details. Values are means $\pm$ S.E.M. of the numbers of determinations indicated in parentheses. Statistical comparisons (Student's *t* test) are: ** $P < 0.01$, *** $P < 0.001$ versus control.

Condition	Na ⁺ -dependent transport	
	(nmol/3 min per mg of protein)	(%)
Control	2.49 $\pm$ 0.15 (4)	100
Control + CLD	2.45 $\pm$ 0.06 (5)	98
Control + CHX	2.43 $\pm$ 0.13 (3)	98
Starved	3.73 $\pm$ 0.12 (5)***	150
Starved + CLD	3.83 $\pm$ 0.20 (5)**	154
Starved + CHX	2.43 $\pm$ 0.16 (3)	98
Starved + sucrose	5.86 $\pm$ 0.29 (3)***	235
Starved + sucrose + CLD	3.35 $\pm$ 0.19 (5)**	134
Starved + sucrose + CHX	2.35 $\pm$ 0.04 (4)	94

sucrose addition, we studied the effect of 10 µg/ml colcemid on both responses. Results are shown in Table 2. Addition of either colcemid or cycloheximide did not modify the basal Na⁺-dependent L-alanine-uptake rates in those cells cultured in a normal medium. When cells were derepressed for 12 h, system A induction was blocked by cycloheximide but was insensitive to colcemid. However, when derepressed cells were further incubated for 12 h more in an amino-acid-free medium supplemented with 200 mM sucrose, as described above a much larger induction was observed. This enhancement was blocked by both cycloheximide and colcemid. These results clearly show that the inductions of system A activity triggered by amino acid starvation or sucrose treatment differ in their sensitivity to inhibitors of microtubular function. This suggests a role for the microtubular machinery in mediating the effects induced by sucrose.

Effect of sucrose addition on cell volume

The colcemid experiments, along with the evidence that sucrose and mannitol exert similar effects on system A activity, strongly suggest that the physiological effects that we are studying are related to changes in cell volume, induced by the hypertonic medium. The changes in intracellular volume, after sucrose addition to cells that had been cultured either in a normal medium or in an amino-acid-free medium, were measured. Results are shown in Figure 3. Addition of 200 mM sucrose to cells cultured in a normal medium induced rapid shrinking, followed by slower swelling until full recovery of cell volume after 12 h of incubation. However, when cells were first kept for 12 h in an amino-acid-free medium and 200 mM sucrose was then added, we observed that cell shrinking occurred to a larger extent. The recovery of cell volume occurred more slowly; it remained low after 12 h of sucrose addition, and only reached similar values to those found in amino-acid-free medium without sucrose after 24 h (results not shown). Amino acid starvation resulted in a slow but progressive decay in cell volume. These

Figure 3 Changes in intracellular volume of NBL-1 cells after sucrose addition

NBL-1 cell monolayers were processed as indicated in the Experimental section. Intracellular volume was assessed after addition of sucrose at the desired times. Values are averages of the observations on two different cell monolayers which agreed within 10%. Symbols: ●, control cells + sucrose; ■, amino-acid-starved cells; ○, amino-acid-starved cells + sucrose.

results suggest that amino acids could play a role in modulating cell volume responses.

DISCUSSION

In this study we show that system A activity which has already been derepressed by amino acid starvation is induced when confluent NBL-1 cell monolayers are incubated with high concentrations of either sucrose or mannitol. This induction is associated with volume regulatory mechanisms. Hypertonicity causes cell shrinking and swelling, but the magnitude and lag time of the effect depends on the presence of amino acids in the incubation medium. A role for cytosolic Na^+ in controlling cell volume has been suggested by others (Hebert, 1986; Haüssinger and Lang, 1991a,b; Preising and Alpern, 1991). In fact, recovery of cell volume after a hypertonic shock seems to be caused by a net increase in intracellular NaCl concentrations, probably mediated by an enhancement of the activity of the Na^+/H^+ antiporter and subsequent activation of the $\text{HCO}_3^-/\text{Cl}^-$ exchanger (Haüssinger and Lang, 1991a). Thus we cannot rule out the possibility that the Na^+/H^+ antiporter is involved in the induction of system A, although we have not yet studied this specific point. However, other Na^+ -transport systems could probably contribute to the volume-regulatory response. Indeed, it has been reported that the addition of system N substrates increase liver cell volume (Baquet et al., 1990; Bode and Kilberg, 1991). In accordance with this view, we show that extracellular amino acids determine cell volume and the ability of cells to respond to a hypertonic shock. Indeed, amino acid starvation by itself causes a progressive decay in cell volume, and cells cultured in amino-acid-free media are known to have lower intracellular Na^+ concentrations (Qian et al., 1991).

A role for cell volume in regulating certain physiological functions has been suggested by others. In rat skeletal muscle, amino acid release is regulated by changes in osmolarity of the medium (Parry-Billings et al., 1990). In rat hepatocytes, stimulation of glycogen synthesis by amino acids seems to be due to cell swelling resulting from Na^+ -dependent amino acid uptake (Baquet et al., 1990). Furthermore, Haüssinger and co-workers have reported a great deal of evidence showing that the volume of liver parenchymal cells is under hormonal control and that

changes in cell size might act as 'second messengers' of hormonal action (Haüssinger and Lang, 1991a; vom Dahl et al., 1991a,b). A main target for this effector seems to be the proteolytic pathway(s) (Hallbrucker et al., 1991; Haüssinger and Lang, 1991a; vom Dahl et al., 1991a). It has been shown recently that system N is also under cell volume control (Bode and Kilberg, 1991). Indeed, cell swelling caused by amino acid addition or by hypotonic media enhances system N activity in cultured rat hepatocytes. A common feature of all these effects is that they are rapid and independent of protein synthesis, although, as indicated above, long-term effects triggered by anisotonic media have been also reported. In the cell line PAP-HT25 derived from rabbit renal inner medulla, hyperosmolarity induces the accumulation of the mRNA coding for aldose reductase, a key enzyme in the biosynthesis of sorbitol, an organic osmolyte (García-Pérez et al., 1989). Furthermore, the effect of some system A and N substrates on ODC induction reported in different established cell lines (Rinehart and Canellakis, 1985; Rinehart et al., 1985) may be also mediated by changes in cell volume, because amino acids by themselves may cause cell swelling (Baquet et al., 1990; Bode and Kilberg, 1991), and it has been shown that a sudden decrease in ambient osmolality is followed by an enhancement of ornithine decarboxylase activity in L1210 cells (Poulin and Pegg, 1990). Other reports suggested that an anisotonic shock could modulate amino acid transport by long-term mechanisms (Klip et al., 1986; Petronini et al., 1990; for review see Haüssinger and Lang, 1991b). In our study, we report a long-term effect, dependent on protein synthesis and probably related to a decrease in cell volume. It has been described that hyperosmolarity caused by NaCl increased system A activity in thymocytes in a partially protein-synthesis-dependent manner (Klip et al., 1986). However, it is known that NaCl addition may directly modify the membrane potential and Na^+/K^+ -ATPase activity (Moule and McGivan, 1990). Furthermore, it has been reported that system A activity is induced in chick-embryo fibroblasts, when these are cultured in media isotonicity supplemented with disaccharide (Petronini et al., 1990). These authors suggest that induction of system A is associated with a volume-regulatory process which should converge at some step with the derepression transduction signal.

The established bovine renal epithelial cell line NBL-1 is a good model to dissect both mechanisms. These confluent cell monolayers can only express system A activity when incubated in derepressed conditions (amino-acid-free media). Indeed, the system A induction obtained after 12 h of amino acid starvation is increased by sucrose addition, and this second increase in system A activity is additive to the previous one. Furthermore, the derepression response is not blocked by colcemid addition, whereas induction by sucrose treatment is. This finding is consistent with a change in cell volume and a role for microtubular function in mediating either system-A gene expression, insertion of new carriers into the plasma membrane, or both. These data, taken together, would suggest that different mechanisms mediate these two effects. Indeed, it seems that expression of system A by adaptive regulation is a prerequisite to obtain an effective response to sucrose treatment, because cells which had been grown in a normal medium did not express system A when sucrose was added.

These observations suggest a novel mechanism of regulation of amino acid transport systems, which can be of physiological relevance in those kidney epithelial cells subjected to hypertonic media.

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