

## Hypertonic Stress and Amino Acid Deprivation Both Increase Expression of mRNA for Amino Acid Transport System A

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The activity of amino acid transport system A (Oxender and Christensen, 1963) is regulated in a variety of different ways, the best studied being the increases of its activity caused by starving cells of amino acids or by exposing them to hypertonicity (for review see McGivan and Pastor-Anglada, 1994). Recently, López-Fontanals et al. (2003) reported in the *Journal of General Physiology* that hypertonic activation of system A in Chinese hamster ovary (CHO-K1) cells, in contrast to its activation by amino acid deprivation, did not involve increased transcription of the mRNA for one of the system A isoforms. We shall follow the suggestion of Mackenzie and Erickson (2004) and call the isoform SNAT2, for sodium-coupled neutral amino acid transporter 2, instead of ATA2 or SAT2 or SA1. This finding supported a scheme, proposed before the cloning of system A, that features basically different mechanisms of response to these two stresses. The scheme, based on work with CHO-K1 and kidney epithelial (NBL-1) cells, suggests the response to amino acid starvation involves increased synthesis of system A transporters, whereas the response to hypertonicity involves the synthesis of another protein that activates existing system A transporters (Ruiz-Montasell et al., 1994). Unfortunately, however, this neat picture cannot obviously be reconciled with several previous studies, albeit with different cells, that gave contradictory results.

There is no problem with the conclusion that the response to amino acid deprivation (also known as “adaptive regulation”) involves increased expression of SNAT2 mRNA. This is consistent with all other reports. For example, amino acid deprivation was shown to increase the abundance of SNAT2 mRNA in cultured human fibroblasts (Franchi-Gazzola et al., 2001), rat C6 glioma cells (Ling et al., 2001), murine T lymphocytes (Trama et al., 2002), and human hepatoma (HepG2) cells (Bain et al., 2002). In both L6 myotubules and 3T3-L1 adipocytes, an increase in abundance of SNAT2

protein followed amino acid deprivation (Hyde et al., 2001). On the other hand, there is no other report that agrees with the different basic response to hypertonicity. In contrast, hypertonicity was found to cause an increase in the amount of SNAT2 mRNA in porcine endothelial cells (Alfieri et al., 2001, 2002), murine inner medullary collecting duct (mIMCD3) cells (Nahm et al., 2002), rat blood brain barrier (TR-BBB13) cells (Takanaga et al., 2002), and murine T lymphocytes (Trama et al., 2002). Since it seemed unlikely to us that the same signal (hypertonicity) activates the same isoform of system A (SNAT2) via a fundamentally different mechanism in CHO-K1 cells, we have checked some of these results. As shown and discussed below, we find that hypertonic stress, like amino acid starvation, does cause an increase in the abundance of SNAT2 mRNA in CHO-K1 cells, as well as in others.

Apart from the use of different cells, details of our materials and methods were as described in recent papers (Alfieri et al., 2001, 2004). CHO-K1 cells were provided by the American Type Culture Collection and obtained through the Istituto Zooprofilattico Sperimentale (Brescia, Italy). They were maintained in MEM supplemented with 10% FCS, 1 mM sodium pyruvate, and a mixture of nonessential amino acids. A cDNA probe for human SNAT2 (Sugawara et al., 2000) was supplied by V. Ganapathy (Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA).

We found that hypertonic (0.5 Osm) incubation of CHO-K1 cells increased the activity of system A in a manner similar to that noted for other cells examined under similar conditions, with a peak of activity around 9 h followed by a fairly rapid decrease toward the control value. This pattern depends on the concentrations of compatible osmolytes, such as betaine and *myo*-inositol, in the incubation medium (Alfieri et al., 2002). Amino

*Abbreviations used in this paper:* CHO, Chinese hamster ovary; SNAT2, sodium-coupled neutral amino acid transporter 2.

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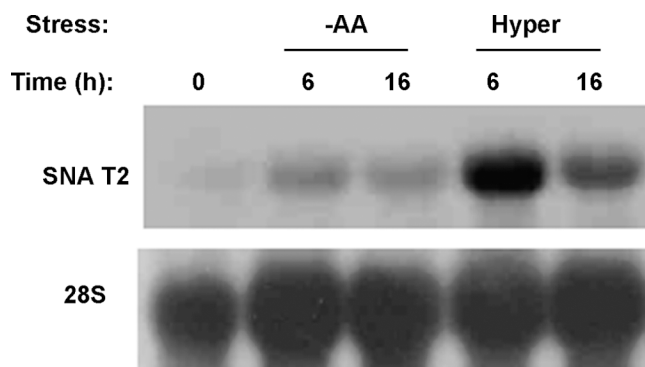


FIGURE 1. Induction of SNAT2 mRNA in CHO-K1 cells. CHO-K1 cells were incubated for 6 or 16 h in medium depleted of amino acids (-AA) or made hypertonic (0.5 Osm) by the addition of sucrose (Hyper). Total cellular RNA was then extracted and analyzed for SNAT2 mRNA by Northern blotting. 28S rRNA was used for standardization. Similar results were obtained in three different experiments.

acid starvation caused a smaller, but sustained, increase in activity. Moreover, both kinds of stress, hypertonicity as well as amino acid starvation, caused increases in the abundance of SNAT2 mRNA in CHO-K1 cells in parallel with the increases in transport activity (Fig. 1). Control checks gave very similar results with porcine endothelial cells, MDCK cells, and human WI-38 fibroblasts, consistent with the notion that these responses are general.

There is no obvious explanation for the difference between our results with CHO-K1 cells and those of López-Fontanals et al. (2003). Differences in the cDNA probes used in these two studies cannot be responsible for the lack of agreement because the probe used by López-Fontanals et al. did give a positive result with CHO-K1 cells in response to amino acid starvation. Hence, some other undetected technical reason seems most likely. Whatever the explanation, it is difficult to obtain a false positive Northern blot, and our result with CHO-K1 cells, if assumed to be correct, removes the only discrepant finding about the response of system A to hypertonic stress. All other previous observations, as noted above, agree that the increase in activity of system A in cells exposed to hypertonicity is accompanied by an increase in abundance of SNAT2 mRNA. It follows from this conclusion that the signal transduction pathways for hypertonic stress and amino acid starvation must converge at, or before, transcription of the message for SNAT2. Hence the mechanistic scheme proposed by López-Fontanals et al. (2003) is not compatible with our findings, even for CHO-K1 cells, and clearly cannot be applicable in any of the other cells studied.

These observations do not preclude the possibility that the initial, immediate, activation of SNAT2 by either hypertonic stress or amino acid deprivation could

occur via recruitment to the plasma membrane of transporter molecules already present in intracellular membranes. Indeed, there is already good evidence that this is the immediate response in rat C6 glioma cells to amino acid starvation, before increased synthesis of SNAT2 (Ling et al., 2001). It seems likely that such initial recruitment also occurs in other cells subject to amino acid deprivation, and it might well occur similarly in response to hypertonic stress.

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#### REFERENCES

- Alfieri R.R., P.G. Petronini, M.A. Bonelli M, A.E. Caccamo, A. Cavazzoni, A.F. Borghetti, and K.P. Wheeler. 2001. Osmotic regulation of ATA2 mRNA expression and amino acid transport System A activity. *Biochem. Biophys. Res. Commun.* 283:174–178.
- Alfieri, R.R., A. Cavazzoni, P.G. Petronini, M.A. Bonelli, A.E. Caccamo, A.F. Borghetti, and K.P. Wheeler. 2002. Compatible osmolytes modulate the response of porcine endothelial cells to hypertonicity and protect them from apoptosis. *J. Physiol.* 540: 499–508.
- Alfieri, R.R., P.G. Petronini, M.A. Bonelli, S. Desenzani, A. Cavazzoni, A.F. Borghetti, and K.P. Wheeler. 2004. Roles of compatible osmolytes and heat shock protein 70 in the induction of tolerance to stresses in porcine endothelial cells. *J. Physiol.* 555:757–767.
- Bain, B.J., R. LeBlanc-Chaffin, H. Chen, S.S. Palii, K.M. Leach, and M.S. Kilberg. 2002. The mechanism for transcriptional activation of the human ATA2 transporter gene by amino acid deprivation is different than that for asparagine synthetase. *J. Nutr.* 132:3023–3029.
- Franchi-Gazzola, R., R. Sala, O. Bussolati, R. Visigalli, V. Dall'Asta, V. Ganapathy, and G.C. Gazzola. 2001. The adaptive regulation of amino acid transport system A is associated to changes in ATA2 expression. *FEBS Lett.* 490:11–14.
- Hyde, R., G.R. Christie, G.L. Litherland, E. Hajdich, P.M. Taylor, and H.S. Hundal. 2001. Subcellular localization and adaptive up-regulation of the System A (SAT2) amino acid transporter in skeletal-muscle cells and adipocytes. *Biochem. J.* 355:563–568.
- Ling, R., C.C. Bridges, M. Sugawara, T. Fujita, F.H. Leibach, P.O.D. Prasad, and V. Ganapathy. 2001. Involvement of transporter recruitment as well as gene expression in the substrate-induced adaptive regulation of amino acid transporter system A. *Biochim. Biophys. Acta.* 1512:15–21.
- López-Fontanals, M., S. Rodríguez-Mulero, F.J. Casado, B. Dérjard, and M. Pastor-Anglada. 2003. The osmoregulatory and the amino acid-regulated responses of system A are mediated by different signal transduction pathways. *J. Gen. Physiol.* 122:5–16.
- Mackenzie, B., and J.D. Erickson. 2004. Sodium-coupled neutral amino acid (system N/A) transporters of the SLC38 gene family. *Pflugers Arch.* 447:784–795.
- McGivan, J.D., and M. Pastor-Anglada. 1994. Regulatory and molecular aspects of mammalian amino acid transport. *Biochem. J.* 299: 321–334.
- Nahm, O., S.K. Woo, J.S. Handler, and M. Kwon. 2002. Involvement of multiple kinase pathways in stimulation of gene transcription by hypertonicity. *Am. J. Physiol. Cell Physiol.* 282:C49–C58.
- Oxender, D.L., and H.N. Christensen. 1963. Distinct mediating sys-

- tems for the transport of neutral amino acids by the Ehrlich cell. *J. Biol. Chem.* 238:3686–3699.
- Ruiz-Montasell, B., M. Gómez-Angelats, F.J. Casado, A. Felipe, J.D. McGivan, and M. Pastor-Anglada. 1994. Evidence for a regulatory protein involved in the increased activity of system A for neutral amino acid transport in osmotically stressed mammalian cells. *Proc. Natl. Acad. Sci. USA.* 91:9569–9573.
- Sugawara, M., T. Nakanishi, Y.J. Fei, W. Huang, M.E. Ganapathy, F.H. Leibach, and V. Ganapathy. 2000. Cloning of an amino acid transporter with functional characteristics and tissue expression pattern identical to that of system A. *J. Biol. Chem.* 275:16473–16477.
- Takanaga, H., N. Tokuda, S. Ohtsuki, K.I. Hosoya, and T. Terasaki. 2002. ATA2 is predominantly expressed as system A at the blood-brain barrier and acts as brain-to-blood efflux transport for L-proline. *Mol. Pharmacol.* 61:1289–1296.
- Trama, J., Y.G. Go, and S.N. Ho. 2002. The osmoprotective function of the NFAT5 transcription factor in T cell development and activation. *J. Immunol.* 169:5477–5488.