Osmotically inducible uptake of betaine via amino acid transport system A in SV-3T3 cells

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The osmotically inducible uptake of betaine (*NNN*-trimethylglycine) by SV-3T3 cells has been studied and compared with the similar process in MDCK cells. Betaine uptake by SV-3T3 cells could be described in terms of a saturable, Na^+-dependent, component plus a small non-saturable, Na^+-independent, component. Transport was active, producing considerable accumulation of betaine in the cells. After exposure of the cells to hypertonic conditions for 6 h, there was a marked increase in betaine uptake. Kinetic analysis indicated that this increase resulted from an increase in the $V_{max.}$ value of the saturable component, from about 88 to 185 nmol of betaine/5 min per mg of protein, the corresponding K_m values of about 15 and 10 mM not being significantly different. This induction of transport activity was detectable only after about 2 h exposure of the cells to hypertonic medium, closely paralleling an induction of influx of *N*-methylaminoisobutyric acid, and was prevented by the presence of cycloheximide. Betaine influx was markedly inhibited by several neutral amino acids, particularly those transported by system A, such as *N*-methylaminoisobutyric acid and the imino acid proline. A high concentration (25 mM) of betaine also significantly inhibited the uptake of proline by SV-3T3 cells. Although very similar results were obtained with MDCK cells, prolonged exposure of cells to hypertonic conditions revealed distinct differences. When the hypertonic incubation was extended from 6 h to 24 h, betaine transport in SV-3T3 cells either remained the same or decreased, whereas it showed a further marked increase in MDCK cells, and also became sensitive to inhibition by γ-aminobutyric acid. mRNA for the betaine transporter BGT-1 [Yamauchi, Uchida, Kwon, Preston, Brooks Robey, Garcia-Perez, Burg and Handler (1992) J. Biol. Chem. **267**, 649–652] was detectable in MDCK cells exposed to hypertonic medium for 24 h, but not in SV-3T3 cells under any conditions. It is concluded that SV-3T3 cells do not produce a specific inducible transporter analogous to BGT-1, but they can accumulate betaine via the amino acid transport system A.

INTRODUCTION

Recently, Yamauchi et al. (1992) cloned the cDNA encoding an osmotically regulated betaine (*NNN*-trimethylglycine) transporter in Madin–Darby canine kidney (MDCK) cells. Synthesis of this transport protein is induced by incubation of the cells in hypertonic media, and the cells can then accumulate betaine to 1000 times its extracellular concentration. mRNA for this protein is localized in kidney medulla, a mammalian tissue that becomes hypertonic under normal conditions, so both the presence and the regulation of this transporter are consistent with its having a role in normal cell function (Nakanishi et al., 1990). The adaptation of other types of cells to hyperosmotic stress, however, can also be enhanced by the presence of extracellular betaine. For example, characteristic responses of fibroblastic cells to hyperosmotic stress include an inhibition of general protein synthesis, coupled with an increase in amino acid transport activity that is dependent on protein synthesis (Tramacere et al., 1984) and an increased production of heat-shock proteins (Petronini et al., 1986). Each of these responses in SV-3T3 cells can be largely counteracted, and cell survival greatly enhanced, by the addition of sufficient betaine to the hypertonic medium (Petronini et al., 1992, 1993a). Moreover, we recently showed that, when SV-3T3 cells were incubated for 6 h in the presence of 25 mM betaine under hypertonic (0.5 osM) conditions, they accumulated betaine to an intracellular concentration of almost 0.2 M, compared with about 0.07 M found in cells similarly incubated under isotonic (0.3 osM) conditions (Petronini et al., 1993b). Hence the question arises of whether cells other than kidney cells also contain the same, or a similar, osmotically regulated betaine transporter. We have therefore made a detailed study of betaine uptake and accumulation by SV-3T3 cells under isotonic and hypertonic conditions in order to characterize the transporter involved.

EXPERIMENTAL

Materials

[α-^{32}P]dCTP, L-[2-^{3}H]glycine, L-[5-^{3}H]proline, [*methyl*-^{14}C]choline chloride and [*methyl*-^{3}H]choline chloride were obtained from Amersham International, Amersham, Bucks., U.K. Choline oxidase and betaine were bought from Sigma Chemical Co., Poole, Dorset, U.K. Both [^{14}C]betaine and [^{3}H]betaine were prepared, from [^{14}C]choline and [^{3}H]choline respectively, exactly as described in detail previously for [^{14}C]betaine (Petronini et al., 1993b). A plasmid containing full-length BGT-1 cDNA (Yamauchi et al., 1992) was kindly provided by Dr. H. Moo Kwon, Division of Nephrology, The John Hopkins University School of Medicine, Baltimore, MD, U.S.A. Media, fetal-calf serum and antibiotics for culturing the cells were purchased from GIBCO, Grand Island, New York, NY, U.S.A. Reagents for electrophoresis and blotting analysis were obtained from Bio-Rad Laboratories, Richmond, CA, U.S.A.

Cell cultures

Simian virus 40-transformed Balb/c 3T3 cells (clone SV-3T3) were kept in culture for up to 2 months and then discarded.

Abbreviation used: GABA, γ-aminobutyric acid.

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Fresh cultures were started again from frozen stocks. The cells were maintained in Dulbecco's modified Eagle medium containing penicillin (100 units/ml) and streptomycin (100 μg/ml) supplemented with 5% fetal-calf serum. MDCK cells (cell line BS Cl 64) were cultured in Eagle's minimum essential medium containing penicillin (100 units/ml) and streptomycin (100 μg/ml) supplemented with 10% fetal-calf serum. All cultures were kept in an incubator at 37 °C in a water-saturated atmosphere of 5% CO_2 in air, and were passaged twice a week. They were regularly checked for mycoplasma contamination with the use of a mycoplasma-detection kit (Boehringer, Mannheim, Germany). During experiments all cells, SV-3T3 and MDCK, were incubated in media containing 10% fetal-calf serum.

Culture media of altered hypertonicity

Usually hypertonic media were prepared by addition of extra NaCl, but for some experiments, as indicated in the text, either LiCl or choline chloride was used instead. The final osmolarities of the modified media were checked with a vapour-pressure osmometer (Wescor).

Northern blotting

Total RNA was extracted from cultured cells by the guanidinium/caesium trifluoroacetate method (Okayama et al., 1987) with a RNA-extraction kit (Pharmacia). RNA samples (30 μg) were fractionated by 1%-agarose gel electrophoresis and transferred to nylon filters. The quality and quantity of RNA blotted on membranes was checked by u.v. absorption. The linearized plasmid containing full-length BGT-1 cDNA (Yamauchi et al., 1992) was nick-translated (Amersham kit N.5000) with [α-^{32}P]-dCTP (3000 Ci/mmol). For hybridization the membranes were incubated in a solution containing 50% formamide, 7% SDS, 0.25 M NaH_2PO_4/Na_2HPO_4 (pH 7.2), 0.25 M NaCl and 1 mM EDTA. Afterwards they were washed according to the following routine, each solution being buffered at pH 7 and each wash lasting 10 min: five times at room temperature in 2 × SSC solution (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7) containing 0.1% SDS; once with 1 × SSC containing 0.1% SDS at 42 °C; twice with 0.1 × SSC containing 0.1% SDS at 42 °C. Autoradiographs were obtained from exposures of 12–24 h, with intensifying screens, at −80 °C.

Uptake of betaine and amino acids

The accumulation of betaine by the cells was monitored with the use of labelled betaine added to the incubation media and subsequent measurement by scintillation counting of radioactivity in acid extracts of washed cells. For measurements of the rates of uptake of betaine or amino acids, the following procedure was used. After they had been incubated in the appropriate medium, cell monolayers were quickly washed with Earle's basic salt solution containing 0.1% glucose (EBSSG) and then incubated in EBSSG for 20 min at 37 °C to diminish the cellular pool of amino acids. The cells were washed again and immediately incubated at 37 °C for the desired time in EBSSG containing labelled betaine or amino acid. When necessary, Na^+ in the medium was replaced by Li^+ or choline ions. The incubations were stopped by quickly washing the cells with cold EBSSG, after which they were extracted with 10% trichloroacetic acid. Radioactivity in samples of the acid extracts was measured by scintillation counting. Cell protein, precipitated by the trichloroacetic acid, was dissolved in 0.2 M NaOH, and its concentration was determined by a dye-fixation method (Bio-Rad) with BSA as standard (Bradford, 1976).

RESULTS

Uptake and accumulation of betaine by SV-3T3 cells

A previous experiment showed that about 80% of betaine uptake by SV-3T3 cells during a 5 min incubation was Na^+-dependent (Petronini et al., 1993b). It was therefore possible that the markedly enhanced accumulation of betaine by cells incubated for 6 h in hypertonic (0.5 osM), compared with isotonic (0.3 osM), medium could have been caused simply by the increased concentration of NaCl used to make the medium hypertonic (Petronini et al., 1993b). To test this possibility, three hypertonic media were made by the addition of different salts, NaCl, LiCl or choline chloride, to the usual isotonic medium, and betaine accumulation by the cells during a 6 h incubation in these media with 25 mM betaine was again measured. In isotonic (0.32 osM) medium the cells accumulated 386±9 nmol of betaine/mg of protein. In hypertonic (0.52 osM) media containing added NaCl, LiCl or choline chloride, the accumulations were 937±43, 900±78 and 855±10 nmol of betaine/mg of protein respectively (mean values±S.D. of 3 measurements; results are given in terms of mg protein because cell volumes were not simultaneously measured). Hence it is clear that the increased accumulation of betaine occurs during incubation under hypertonic conditions whether or not NaCl is used as the extra osmolyte. (These results also indicate that choline itself does not significantly affect betaine accumulation, so that choline chloride may be used to replace NaCl for determination of the effect of Na^+ ions on betaine uptake). We therefore compared the rates of uptake of betaine, measured under standard conditions, by cells that had previously been incubated for 6 h under either isotonic or hypertonic conditions.

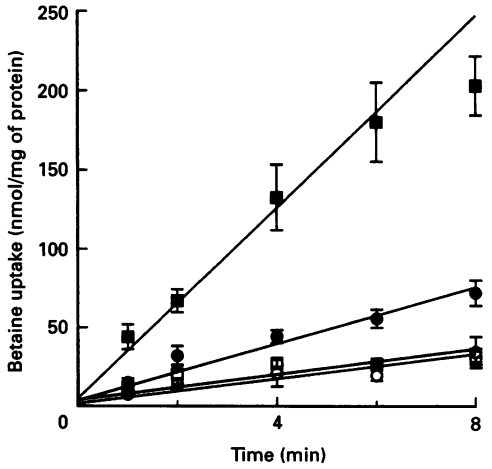

Figure 1 Hypertonic incubation stimulates the rate of Na^+-dependent betaine uptake

SV-3T3 cells were first incubated for 6 h in isotonic (0.3 osM) medium (control cells) or hypertonic (0.5 osM) medium (test cells). Then all samples were incubated under the standard conditions described in the Experimental section, first to deplete cells of intracellular amino acids and then with 10 mM labelled betaine to measure betaine influx. Na^+-independent betaine uptake was determined with the use of medium in which all Na^+ ions had been replaced with choline ions. Key: ●, control cells in the presence of Na^+; ○, control cells in the absence of Na^+; ■, test cells in the presence of Na^+; □, test cells in the absence of Na^+. Mean values (±S.D.) from 3 measurements are given.

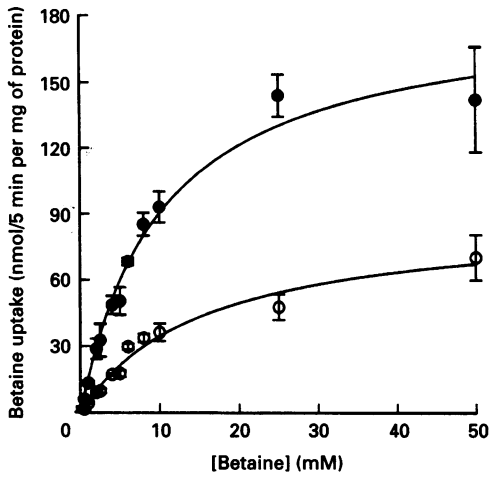

Figure 2 Kinetics of betaine uptake

Test and control SV-3T3 cells were prepared as described in the legend to Figure 1 and then their initial rates of betaine uptake were measured in the absence and presence of Na^+ and with a range of betaine concentrations. Mean values (±S.E.M.) for the Na^+-dependent influx are given for 3–7 measurements at each betaine concentration from three separate experiments. The curves, drawn with the use of the Fig-P curve-fitter program (Biosoft), fit equations with kinetic parameters $K_m = 15.5 \pm 2.3$ mM and $V_{max.} = 88 \pm 6$ nmol/5 min per mg of protein for control cells (○), and $K_m = 10.4 \pm 1.2$ mM and $V_{max.} = 185 \pm 9$ nmol/5 min per mg of protein for test cells (●).

Figure 1 shows the time course of uptake of betaine, measured in the presence or absence of Na^+, by cells previously incubated under the two different osmotic conditions. Uptake was linear with time for at least 6 min, even with the relatively high concentration (10 mM) of betaine used, was clearly faster in the presence of Na^+, and the Na^+-dependent component was markedly faster in cells previously exposed to hypertonic conditions. Thus the increased accumulation of betaine seen during hypertonic incubations is caused by an increased rate of Na^+-dependent uptake. These results also showed that a good approximation of the initial rate of uptake of betaine could be obtained with the use of a 5 min incubation.

Kinetics of betaine uptake

The variation in the rate of betaine influx as a function of extracellular betaine concentration was measured, and the data were analysed with the use of the Fig-P curve-fitter programme (Biosoft) in terms of a non-saturable, Na^+-independent, component of influx plus a saturable, Na^+-dependent, component. The results are shown in Figure 2. For control cells, previously incubated in isotonic (0.3 osM) medium for 6 h, the best-fit equation for the saturable component corresponded to values of 15.5 ± 2.3 mM for K_m and 88 ± 6 nmol of betaine/5 min per mg of protein for $V_{max.}$. For cells previously incubated in hypertonic (0.5 osM) medium for 6 h, the corresponding values were 10.4 ± 1.2 mM for K_m and 188 ± 9 nmol/5 min per mg for $V_{max.}$. Hence the clear findings are that the Na^+-dependent component of betaine influx has a high K_m value, of the order of 10–15 mM, and that its $V_{max.}$ value significantly increases after incubation of the cells in hypertonic medium. This K_m value of 10–15 mM for betaine uptake has to be compared with a value of about 0.6 mM for the K_m of Na^+-dependent influx of proline into these cells (P. G. Petronini, E. De Angelis, A. F. Borghetti and K. P. Wheeler, unpublished work).

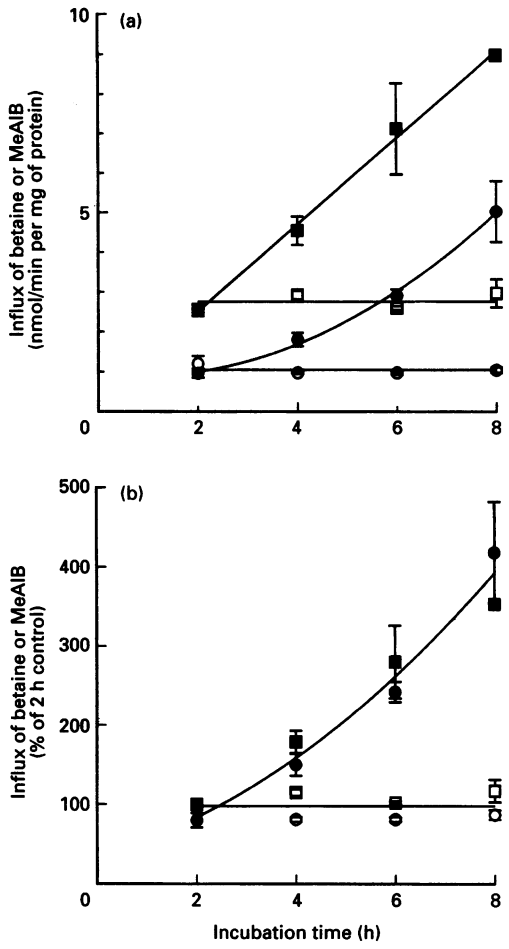

Figure 3 Time course of induction of transport activity

(**a**) SV-3T3 cells were first incubated for the indicated periods of time in isotonic (0.3 osM) or hypertonic (0.5 osM) medium. Then they were washed, and their rates of uptake of betaine and *N*-methylaminoisobutyric acid (MeAIB) were measured under the standard conditions described in the Experimental section, with incubation for 5 min for betaine and for 1 min for MeAIB, each being present separately at 0.1 mM. Mean values (±S.D.) from 3 measurements are given. (**b**) Data from (**a**) expressed as percentages of the 2 h control values. Key: ○, □, control cells; ●, ■, test cells; ○, ●, betaine; □, ■, MeAIB.

Induction of betaine-uptake activity during incubation of SV-3T3 cells under hypertonic conditions

The results in Figure 3 show that the increase in the initial rate of betaine influx occurred only after the cells had been exposed to hypertonic conditions for about 2 h, paralleling the increase in influx of *N*-methylaminoisobutyric acid, which is usually accepted to be a 'model substrate' for amino acid transport system A (Christensen et al., 1965; Shotwell and Oxender, 1983). No such induction of betaine uptake activity occurred when cycloheximide was present in the hypertonic medium, whereas betaine uptake by control cells (incubated in isotonic medium) was unaffected by the addition of cycloheximide (Table 1), indicating that the induction depended on protein synthesis. Since these findings closely parallel those recorded previously for the hypertonic induction of amino acid transport activity via system A in chick-embryo fibroblasts (Tramacere et al., 1984; Petronini et al., 1990), it seemed possible that betaine uptake by SV-3T3 cells could be occurring via system A.

Table 1 Prevention by cycloheximide of induction of betaine uptake

SV-3T3 cells were incubated for 6 h in isotonic (0.3 osM) or hypertonic (0.5 osM) media in the absence or presence of cycloheximide (10 μg/ml). After they had been washed and incubated under standard conditions, to deplete cellular amino acid pools, the cells were then incubated for 5 min with 0.1 mM [^{3}H]betaine for measurement of betaine influx, as described in the Experimental section. Mean values (±S.D.) from 3 measurements are given.

	Betaine influx (nmol/5 min per mg of protein) into cells previously incubated in:	
Initial conditions	Isotonic medium	Hypertonic medium
Control	0.56±0.08	1.01±0.04
Plus cycloheximide	0.55±0.05	0.60±0.05

Inhibition of betaine influx by amino acids

To check the interpretation suggested above, that betaine uptake by the SV-3T3 cells could in fact be via amino acid transport system A, we tested the ability of a number of amino acids to inhibit betaine influx. The results (Table 2) revealed that betaine influx was indeed inhibited by most of the amino acids tested, in both normal cells and those previously incubated in hypertonic medium. Moreover, the most potent inhibitor under the conditions used was *N*-methylaminoisobutyric acid, whilst other typical 'A-system substrates', serine and proline, also inhibited considerably more effectively than did leucine and phenylalanine, which are typical 'L-system substrates'.

Inhibition of amino acid influx by betaine

Since the transport of both betaine and amino acids by the same transport system should result in their mutual competition, we also tested the effect of extracellular betaine as an inhibitor of uptake via amino acid transport system A. Proline was chosen as a good example of a natural imino acid transported via the Na^+-dependent system A, and a high concentration (25 mM) of betaine was used because the kinetic analysis (Figure 2) indicated that the K_m value for betaine uptake is very high. Glycine was also used, because betaine may be regarded as a derivative of it. The results in Table 3 show that, under these conditions, proline influx into both control and test cells was significantly inhibited by the betaine, as was glycine uptake by test cells. Glycine influx into control cells, however, was not inhibited. Comparison of the results in Tables 2 and 3 shows that, quantitatively, the amino acids are much more potent inhibitors of betaine uptake than vice versa.

Comparison with MDCK cells

Several tests were made to compare the osmotically inducible transport of betaine in SV-3T3 more directly with that in MDCK cells. Examination of the extent of induction of transport activity as a function of time revealed a clear difference. For example, in

Table 2 Inhibition of betaine uptake by amino acids

SV-3T3 cells were first incubated for 6 h in isotonic (0.3 osM) medium (control cells) or hypertonic (0.5 osM) medium (test cells). Then all samples were incubated under the standard conditions described in the Experimental section, first to deplete cells of intracellular amino acids and then with labelled betaine to measure betaine influx. The extracellular concentration of betaine was 0.1 mM, and influx was measured during a 5 min incubation in the presence of Na^+. The indicated amino acids were present in the medium used for the influx measurements at a final concentration of 10 mM. Abbreviation: MeAIB, *N*-methylaminoisobutyric acid. Mean values (±S.D.) from 3 measurements are given.

		Betaine influx (nmol/5 min per mg of protein)			
	Addition	Control cells	(% inhibition)	Test cells	(% inhibition)
(a)	None	0.74±0.11	(0)	1.29±0.05	(0)
	MeAIB	0.07±0.01	(91)	0.09±0.01	(93)
	Serine	0.18±0.02	(76)	0.28±0.03	(78)
	Leucine	0.30±0.01	(59)	0.66±0.05	(49)
	Phenylalanine	0.43±0.06	(42)	0.84±0.05	(35)
(b)	None	0.63±0.11	(0)	1.64±0.23	(0)
	Proline	0.16±0.01	(75)	0.28±0.04	(83)
(c)	None	0.39±0.02	(0)	1.48±0.21	(0)
	Glycine	0.13±0.01	(67)	0.44±0.06	(70)

Table 3 Effect of betaine on amino acid influx

These experiments were performed exactly as described in the legend to Table 2, except that amino acid influx was measured during 1 min incubations with 0.1 mM [^{3}H]glycine or [^{3}H]proline, in the presence and absence of 25 mM betaine. Mean values (±S.D.) from 3 measurements are given.

		Influx of amino acid (nmol/min per mg of protein)			
Amino acid	Addition	Control cells	(% inhibition)	Test cells	(% inhibition)
Glycine	None	0.85±0.09	(0)	1.68±0.22	(0)
Glycine	Betaine	0.91±0.18	(0)	0.93±0.13	(45)
Proline	None	2.51±0.40	(0)	6.94±0.69	(0)
Proline	Betaine	1.25±0.09	(50)	2.61±0.24	(62)

Table 4 Comparison of SV-3T3 with MDCK cells

(**a**) Control cells were incubated first in isotonic (0.3 osM) medium and test cells in hypertonic (0.5 osM) medium, for 8 or 24 h, as indicated. Betaine uptake by the washed cells was then measured with the use of 0.1 mM [^{3}H]betaine and 5 min incubations as described in the Experimental section. Mean values (±S.D.) from 3 measurements are given. (**b**) As in (**a**), except that the shorter preliminary incubation in hypertonic medium was for 6 h. GABA and proline were added as indicated (final concn. 10 mM) only during measurement of betaine influx. Mean values (±S.E.M.) from the indicated number of measurements are given, the data being collected from 3 separate experiments. *†‡ and ¶ indicate pairs that are significantly different ($P < 0.01$) by Student's t test.

	Cells	Preliminary incubation (h)	Addition	Betain influx (nmol/5 min per mg of protein) Control cells	Test cells
(a)	SV-3T3	8		0.66±0.07	2.51±0.19
		24		0.64±0.04	2.84±0.37
	MDCK	8		0.28±0.07	1.22±0.13
		24		0.31±0.04	4.01±0.42
(b)	MDCK	6	None	0.27±0.03 (9)	0.65±0.03 (9)*†
			GABA	0.23±0.01 (6)	0.68±0.05 (6)
			Proline	0.27±0.04 (6)	0.42±0.02 (6)†
		24	None	0.20±0.04 (9)	2.68±0.31 (9)*‡¶
			GABA	0.16±0.02 (6)	0.34±0.02 (6)‡
			Proline	0.15±0.02 (6)	0.87±0.08 (6)¶

the experiment recorded in Table 4(a), maximum betaine transport activity had been attained in SV-3T3 cells after 8 h of exposure to hypertonic conditions, and there was no further change during the following 16 h. (In some other experiments, betaine-transport activity tended to decrease after such long incubations of SV-3T3 cells.) In contrast, MDCK cells showed not only a marked induction of betaine-transport activity after 8 h of incubation, but also a further, larger, increase during the subsequent 16 h in hypertonic medium.

Another clear difference was shown by comparison of the action of γ-aminobutyric acid (GABA) on betaine influx with the two types of cells. No inhibition of GABA of betaine influx into SV-3T3 cells could be demonstrated under any conditions (results not shown). With MDCK cells that had been exposed to hypertonic medium for only 6 h, GABA similarly had no significant effect on betaine uptake (Table 4b). After 24 h of incubation in hypertonic medium, however, MDCK cells showed a large induced uptake of betaine that was inhibited by 87% by GABA.

The results in Table 4(b) also show that proline, in contrast with GABA, significantly inhibited betaine influx into MDCK cells that had been exposed to hypertonic conditions for only 6 h (35% inhibition), as well as for 24 h (68% inhibition). Since proline is transported by both system A and BGT-1, these observations, taken together with those for GABA, suggest that betaine is also transported by both systems in MDCK cells. (The lack of significant effect of proline on the low influx of betaine into control MDCK cells suggests that system A activity is very low in these cells under control conditions.)

Yamauchi et al. (1992) also reported that the inducible betaine transport in MDCK cells was Cl^--dependent. We have not tried to confirm this finding; but replacement of Cl^- with gluconate in the medium used for influx measurements had no effect on betaine uptake by SV-3T3 cells.

Finally, Figure 4 shows that mRNA coding for the inducible betaine transporter BGT-1 (Yamauchi et al., 1992) was just detectable in MDCK cells after 8 h of exposure to hypertonic conditions, and clearly present after 24 h of treatment. In contrast, none was detectable in SV-3T3 cells after either 8 h or 24 h of incubation in hypertonic medium.

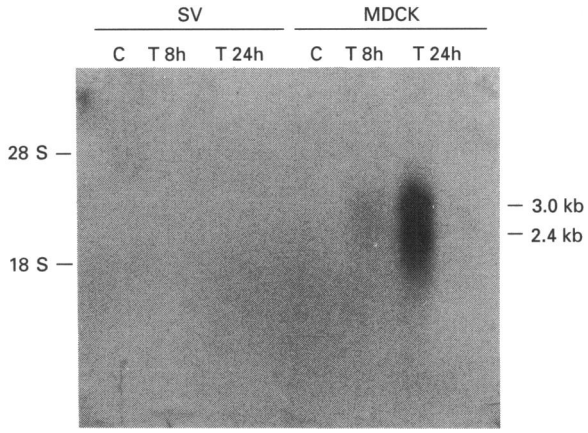

Figure 4 Expression of mRNA for BGT-1

SV-3T3 and MDCK cells were incubated exactly as described in the legand to Table 4, and then total cellular RNA was extracted and analysed for the detection of BGT-1 mRNA by Northern blotting, as described in the Experimental section. Key: C, control cells, incubated in isotonic (0.3 osM) medium; T, test cells, incubated in hypertonic (0.5 osM) medium.

DISCUSSION

The obvious interpretation of the results presented above is that the osmotically inducible uptake of betaine by SV-3T3 cells occurs via the Na^+-dependent A system for amino acid transport, rather than by a system more specific for betaine. Previously, before we had labelled betaine and were unable to analyse the kinetics of its uptake, this explanation seemed unlikely, because of the relative difficulty of demonstrating significant inhibition of amino acid uptake by betaine. The estimated K_m value of the order 10–15 mM for Na^+-dependent uptake of betaine by SV-3T3 cells, compared with the value of about 0.6 mM for proline influx, provides an explanation for the quantitative differences in the mutual inhibitory effects of betaine and the amino acids. The demonstration that betaine is actively transported, and that the Na^+ concentration gradient appears to provide the driving force, are also both in keeping with accepted concepts of the nature of

amino acid transport via system A. In view of the observations made previously of the ability of betaine to counteract the effects of exposure of chick-embryo fibroblasts, 3T3 cells and SV-3T3 cells to hypertonic conditions (Petronini et al., 1990, 1992, 1993a,b), we conclude that betaine uptake occurs similarly via system A in all three types of cell. This conclusion may also apply to amino acid transport system A in general. For example, in some early studies with Ehrlich ascites-tumour cells, Christensen et al. (1965) estimated the K_m value for betaine uptake to be of the order 24–45 mM and suggested that 'most of the uptake of betaine by the Ehrlich cell... may well be accounted for by the alanine-preferring system.' Similarly, our findings with MDCK cells are readily explicable in terms of betaine's being taken up initially, during 6–8 h exposure of the cells to hypertonic conditions, via induced system A activity, and only subsequently via the BGT-1 transporter, which is induced later.

It is interesting that system A appears to be particularly susceptible to induction in response to exposure of cells not only to hypertonic stress, but also to amino acid starvation (Guidotti et al., 1978; Shotwell and Oxender, 1983) and, with lymphocytes, to mitogenic lectins (Borghetti et al., 1979). Although these various different signals seem eventually to converge, the cellular pathways from initial signals to gene expression are unknown. Recently, however, Soler et al. (1993) obtained results indicating that, in the epithelial cell line NBL-1, the effects of adaptive regulation (response to amino acid starvation) and response to hyperosmotic stress are mediated by different mechanisms.

The results presented here also show clearly that the inducible BGT-1 transporter (Yamauchi et al., 1992) is not present in SV-3T3 cells. Obviously it is more specific than amino acid transport system A, although it too is not really specific for betaine, the apparent K_m values for the influx of GABA, betaine and proline (0.1–0.4 mM) being of the same order of magnitude (Yamauchi et al., 1992).

The ability of 'compatible osmolytes' to stabilize proteins under various extreme conditions has attracted intermittent interest over the past two decades, and a recent study compared the effects of glycine, sarcosine, *N N*-dimethylglycine and betaine on the thermal stability of isolated enzymes (Santoro et al., 1992). Each of them provided considerable stabilization against thermal unfolding of the enzymes, but their relative efficacies indicated that, the greater the degree of *N*-methylation, the less effective was the stabilizing effect. Hence, on the basis of this criterion, glycine or sarcosine might be expected to be preferred to betaine as 'compatible osmolytes'. This is contrary to our observations with respect to cell adaptation to hypertonic stress, but the results discussed above provide other reasons for betaine's being a particularly good 'compatible osmolyte' in living cells. First, it is accumulated within the cells to a remarkable extent by the Na^+-dependent uptake mechanism, appearing not to be metabolized (Petronini et al., 1993b). Second, the very low apparent affinity of betaine for the transport system indicates that it does not readily compete with amino acids for uptake. Similarly, it probably does not easily interfere with amino acid metabolism. In contrast, the use of a single amino acid, such as glycine, as a potential 'compatible osmolyte' would obviously result in an imbalance in the amino acid pool within the cells, and thus perturb metabolism. Hence, although induction of amino acid transport system A in response to hypertonic conditions enables the cells partly to counteract the deleterious effects by the uptake of amino acids, the artificial addition of betaine greatly enhances the effectiveness of this response.

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