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Osmotic regulation of taurine transport via system β and novel processes in mouse preimplantation conceptuses

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Abstract

Taurine was shown recently to increase the frequency at which 2-cell mouse conceptuses develop into blastocysts in vitro. For this reason and because taurine helps cells adapt to external stresses, we studied transport of this and related amino acids by preimplantation mouse conceptuses. The most conspicuous component of taurine transport in conceptuses at the 1-cell through blastocyst stages of development was both Na^+- and Cl^--dependent. This Na^+- and Cl^--dependent transport system interacted relatively strongly with β- but not α-amino acids. By these criteria, transport system β is responsible for Na^+-dependent taurine transport in preimplantation mouse conceptuses. Moreover, detection of mRNA encoding the taurine transport protein (TAUT) in early conceptuses supports the theory that TAUT is a major component of system β. Transport of taurine by system β in 1-cell conceptuses was slower in hypotonic than in hypertonic media, whereas the reverse was true for system β in blastocysts. In contrast, hypotonically stimulated Na^+-independent taurine transport was, of course, more rapid in hypotonic than in hypertonic media in both 1-cell conceptuses and blastocysts. Transport via this hypotonically stimulated process also showed no sign of saturation by up to 10 mM taurine. Hypotonically stimulated taurine transport appeared transiently in 1-cell conceptuses under hypotonic conditions until they had recovered their initial volumes. Hence, we suggest that a decrease in taurine uptake via system β and an increase in taurine exodus via the Na^+-independent, nonsaturable transport process could contribute to the regulatory volume decrease in 1-cell conceptuses in hypotonic medium. Since taurine uptake by system β in blastocysts is, however, higher in hypotonic than in hypertonic media, taurine uptake by system β in blastocysts might intensify a tendency to increase cell volume in hypotonic medium. Such an increase in taurine uptake could further favor anabolic changes associated with cell swelling. In addition to contributing to regulation of cellular volume and perhaps metabolism, the hypotonically stimulated Na^+-independent transport processes in early embryos have novel characteristics. Hypotonically stimulated Na^+-independent taurine transport was inhibited by niflumate, *N*-ethylmaleimide and NaN_3 but not by furosemide, iodoacetate, KCN, ouabain or α- or β-amino acids. Furthermore, 4,4′-diisothiocyanostilbene-2,2′-disulfonate inhibited this transport in 1-cell conceptuses but not in blastocysts. Hence, different hypotonically stimulated Na^+-independent taurine transport processes appear to be present in 1-cell conceptuses vs. blastocysts. The functions of these and other instances of developmental regulation of expression of transport processes in preimplantation conceptuses remain largely to be elucidated. Moreover, neither of the hypotonically stimulated Na^+-independent taurine transport processes in conceptuses appears to have been detected in other types of cells. Instead, these processes may be unique to preimplantation conceptuses.

Key words: Taurine transporter; TAUT; Volume regulation; Preimplantation embryo; Insulin; Preimplantation development

1. Introduction

Taurine is used by cells for osmoregulation, modulation of the action of Ca^{2+}, stimulation of Cl^- fluxes and protection against the consequences of plasma membrane damage. Although the mechanisms by which taurine exerts its different effects are still under investigation, a common characteristic of its functions is to help cells respond to external stresses [1]. In this regard, taurine was shown recently to increase the number of two-cell mouse conceptuses that develop into blastocysts in vitro [2]. Blastocysts that develop in the presence of 5 or 10 mM taurine also have more cells

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than those that develop in medium without added taurine. Moreover, early mouse conceptuses normally contain relatively high concentrations of this β-amino acid [3]. Somewhat surprisingly, taurine has been reported not to be transported by preimplantation mouse blastocysts [4].

Since mouse oviductal flushings also contain relatively high concentrations of taurine [2], it is conceivable that high intracellular concentrations of taurine are maintained in early mouse conceptuses by nonmediated diffusion of taurine from the extracellular medium in vitro or oviductal fluid in vivo. On the other hand, taurine is a sulfonic rather than a carboxylic β-amino acid, so the K_a value of its anionic group is 100-fold larger than this value for the corresponding carboxylic acid, β-alanine [1]. Hence, a much smaller proportion of the anionic groups of taurine than of β-alanine would be uncharged at most physiological pH values. Possibly for this reason, diffusion of taurine through the plasma membrane appears to be much slower than diffusion of β-alanine [1]. Because diffusion of taurine into and out of preimplantation conceptuses should be relatively slow, and yet exogenously supplied taurine is beneficial to development of preimplantation mouse conceptuses under some conditions in vitro [2], we reinvestigated mediated taurine transport in preimplantation mouse conceptuses.

Two very different types of mediated taurine transport have been delineated in vertebrate cells (e.g., [1,5–14]). Taurine is taken up by cells against a concentration gradient via the Na^+-and Cl^--dependent β-amino acid transport system. This system interacts relatively strongly with β- but not with α-amino acids, and its activity is apparently down-regulated in some cells by hypoosmotic stress (e.g., [9]). The putative amino acid sequence of one Na^+- and Cl^--dependent taurine transport protein has been determined and found to be homologous to other Na^+- and Cl^--dependent transport proteins including several that transport neurotransmitters (reviewed in [15]). In contrast to system β, hypoosmotic stress and other conditions that lead to cell swelling appear to activate the capacity for taurine uptake or exodus via a Na^+-independent process that resembles a channel. This process appears to select for relatively small amino acids although it is apparently not substrate saturable [9]. Nevertheless, operation of the possible channel appears to be energy-dependent at least in skate hepatocytes [14]. Many vertebrate cells probably adapt to hypotonic stress in part by downregulating taurine uptake via system β and upregulating taurine exodus via the possible channel in order to release taurine. Similarly, these directions of regulation may be reversed in order to accumulate taurine when the extracellular osmolarity becomes hypertonic. Osmotic stresses appear to be tolerated well by preimplantation mouse conceptuses since they seem to develop normally in vitro in media containing a range of osmolarities from less than 250 to more than 350 mosM [16]. For these reasons, we examined preimplantation conceptuses for the presence of system β, a possible channel, and other transporters of β-amino acids.

2. Materials and methods

Preimplantation mouse conceptuses were obtained after induction of ovulation and mating in 8–11-week-old ICR mice (Harlan Sprague Dawley) as described previously (e.g., [17–19]). 1-, 2- and 4–8-cell conceptuses were obtained from the reproductive tract about 19, 43 and 66 h, respectively, after administration of human chorionic gonadotropin. Blastocysts were obtained from the uterus approximately 94 h after injection of this hormone. Conceptuses were washed and stored for 6 h or less in Brinster's medium (MBM) [20] in a humidified atmosphere of 5% CO_2 in air at 37°C (pH 7.4) during which time taurine transport activity was not observed to change. In some cases, blastocysts were collapsed with 10 μg/ml cytochalasin B [21,22] before studying their ability to transport amino acids.

Conceptuses were incubated with a ^3H-labeled form of taurine, β-alanine, L-alanine, or L-valine (17–93 Ci/mmol, Amersham or New England Nuclear) and the indicated nonradioactive substances for 5–60 min at 37°C in a modification (Na^+ salts of lactate and pyruvate replaced with NaCl, Na_2HPO_4 replaced with K_2HPO_4 and Phenol red deleted) of Spindle's [23] flushing medium-I (MFM) [24]. To measure Na^+-independent uptake, Na^+ in the medium was replaced with K^+ or choline. Hypotonically stimulated Na^+-independent transport was measured by diluting Na^+-free MFM in half with water. Both K^+ and choline were used to replace Na^+ in hypotonic medium since K^+ has been used for this purpose in some prior studies (e.g., [9]), and 1-cell conceptuses could recover their initial volumes when choline was used to replace Na^+ (see Results). Although the extent of stimulation of Na^+-independent taurine uptake was somewhat greater in hypotonic medium when choline rather than K^+ was used to replace Na^+, the results of transport experiments were similar in these two media. As discussed extensively in prior publications [24,25], many experiments were performed with substrate concentrations near 1 μM in order to insure that transport activities with relatively low K_m values would be detected without obscuring transport activities that have higher K_m values. This strategy was successful since we detected relatively low K_m, Na^+-dependent taurine transport systems ($K_m = 14$ μM) and nonsaturable ($K_m > 10$ mM) hypotonically stimulated, Na^+-independent transport processes (see Results). Data were analyzed statis-

tically using analysis of variance [26,27] or a nonparametric statistical method when the variances of different groups were significantly different [26].

3. Results

Na^+-dependent transport of β-amino acids

Taurine uptake by conceptuses increased linearly with time for at least 60 min at the 1-cell through blastocyst stages of development (Fig. 1). Most of the latter uptake was Na^+-dependent, and Na^+-dependent transport at 2.9 μM taurine increased significantly between the 4–8-cell and blastocyst stages. Similar results (not shown) were obtained for uptake of β-alanine by 1-cell conceptuses and blastocysts. Na^+-dependent [^{3}H]taurine uptake was inhibited almost completely by 10 mM β-alanine and nonradioactive taurine; less completely by γ-aminobutyrate (GABA), L-alanine and L-arginine; and not in a statistically significant manner by glycine, L-proline, L-leucine or L-aspartate (Table 1). Similar results were obtained for inhibition of uptake of β-alanine, but β-alanine transport in blastocysts was also inhibited incompletely by L-leucine, glycine and L-proline (Table 1). The latter inhibition is attributed to an additional, taurine-resistant component of β-alanine transport in blastocysts (Fig. 2d). The taurine-resistant component of Na^+-dependent β-alanine uptake in blastocysts was inhibited by a broad scope of zwitterionic and cationic amino acids including the bicyclic amino acid, 2-amino*endo*bicyclo[2.2.1]hexane-2-carboxylic acid (BCH), but it was not inhibited in a statistically significant manner by the anionic amino acid, L-aspartate (Table 2).

Table 1
Effect of various amino acids on Na^+-dependent uptake of [^{3}H]taurine or [^{3}H]β-alanine

Amino acid (10 mM)	Uptake (% of control)			
	[^{3}H]taurine (1 μM)		β-[^{3}H]alanine (1 μM)	
	1-cell	blastocyst	1-cell	blastocyst
Control	100±12	100±12	100± 7	100±9
Taurine	4± 1**	2± 1**	12± 1**	16±1**
β-Alanine	5± 1**	3± 1**	10± 1**	8±1**
Glycine	73±14	80± 8	80±14	53±6**
L-Proline	89±12	116±13	101± 8	59±7**
γ-Aminobutyrate	10± 3**	7± 1**	16± 2**	23±5**
L-Alanine	47± 8*	47± 4**	51± 4**	28±3**
L-Leucine	115±23	95± 7	108±11	68±8*
L-Aspartate	93±12	(91)	99± 8	81±7
L-Arginine	50± 8*	(28)	51± 4**	29±4**

Experiments were performed as described in the legend of Fig. 2. Statistically significant inhibition is indicated by single ($P < 0.05$) or double ($P < 0.01$) asterisks except in the case of L-arginine inhibition of [^{3}H]taurine uptake by blastocysts where only two determinations were used to calculate the mean (two determinations also in the case of the effect of L-aspartate). Similar results (not shown) were obtained for inhibition of [^{3}H]taurine uptake by collapsed blastocysts.

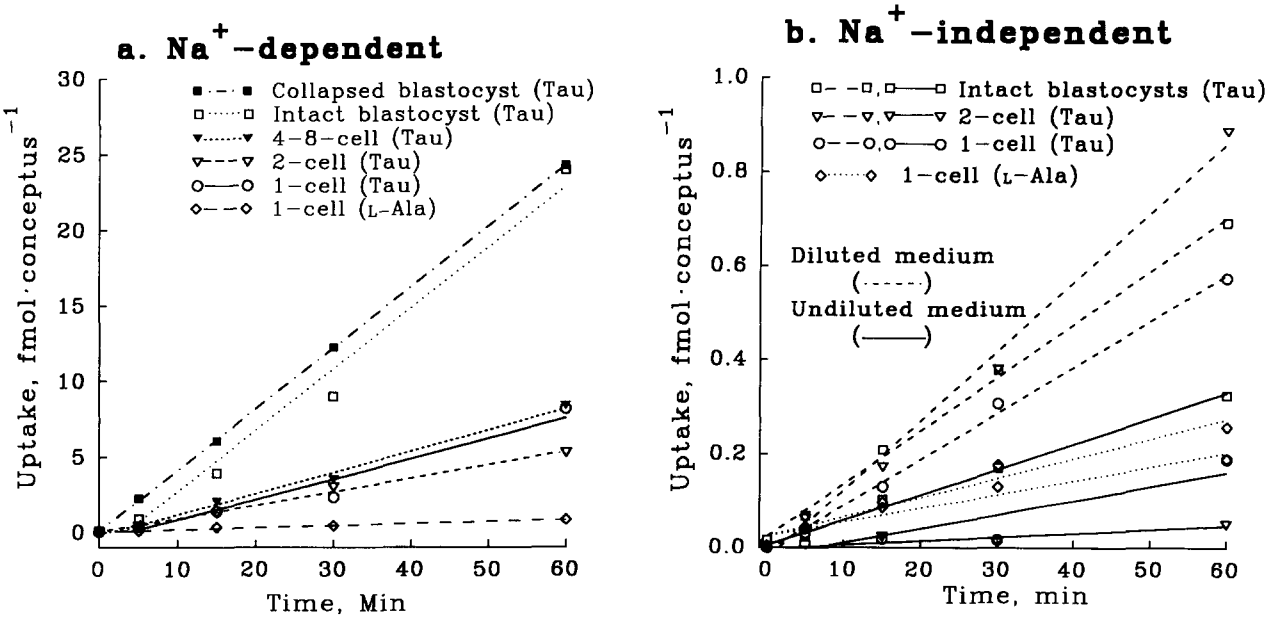

Fig. 1. Uptake of taurine (Tau) and L-alanine by preimplantation mouse conceptuses after various times in the presence or absence of Na^+ and in Na^+-free, hypotonic medium. Conceptuses were incubated for the indicated times with 2.9 μM [^{3}H]taurine (17 Ci/mmol) or 1.0 μM L-[^{3}H]alanine (50 Ci/mmol) in MFM (a), this medium in which Na^+ was replaced with K^+ (solid lines for taurine in b) or this Na^+-free medium diluted in half with water (dashed lines for taurine in b). The top and bottom dotted lines in (b) represent uptake of L-alanine in diluted MFM and undiluted MFM, respectively. Each point represents the mean uptake of 4–7 replicate determinations (3–6 conceptuses/determination) obtained in three or four independent experiments. In general, uptake increased nearly linearly with time for up to 60 min in conceptuses in Na^+-containing or Na^+-free hypotonic medium, and in most of these instances the correlation coefficient was > 0.99.

The taurine-sensitive component of β-alanine uptake in blastocysts and 1-cell conceptuses increased about linearly with the Cl^- concentration, whereas this relationship appeared to be nonlinear for the Na^+ concentration (Fig. 3). Na^+-dependent taurine transport was also Cl^--dependent (data not shown), and it was competitively inhibited by β-alanine (Fig. 4). The kinetics of L-arginine inhibition of taurine transport were complex, and arginine was a much weaker inhibitor than β-alanine (Fig. 4). Interestingly, Na^+-de-

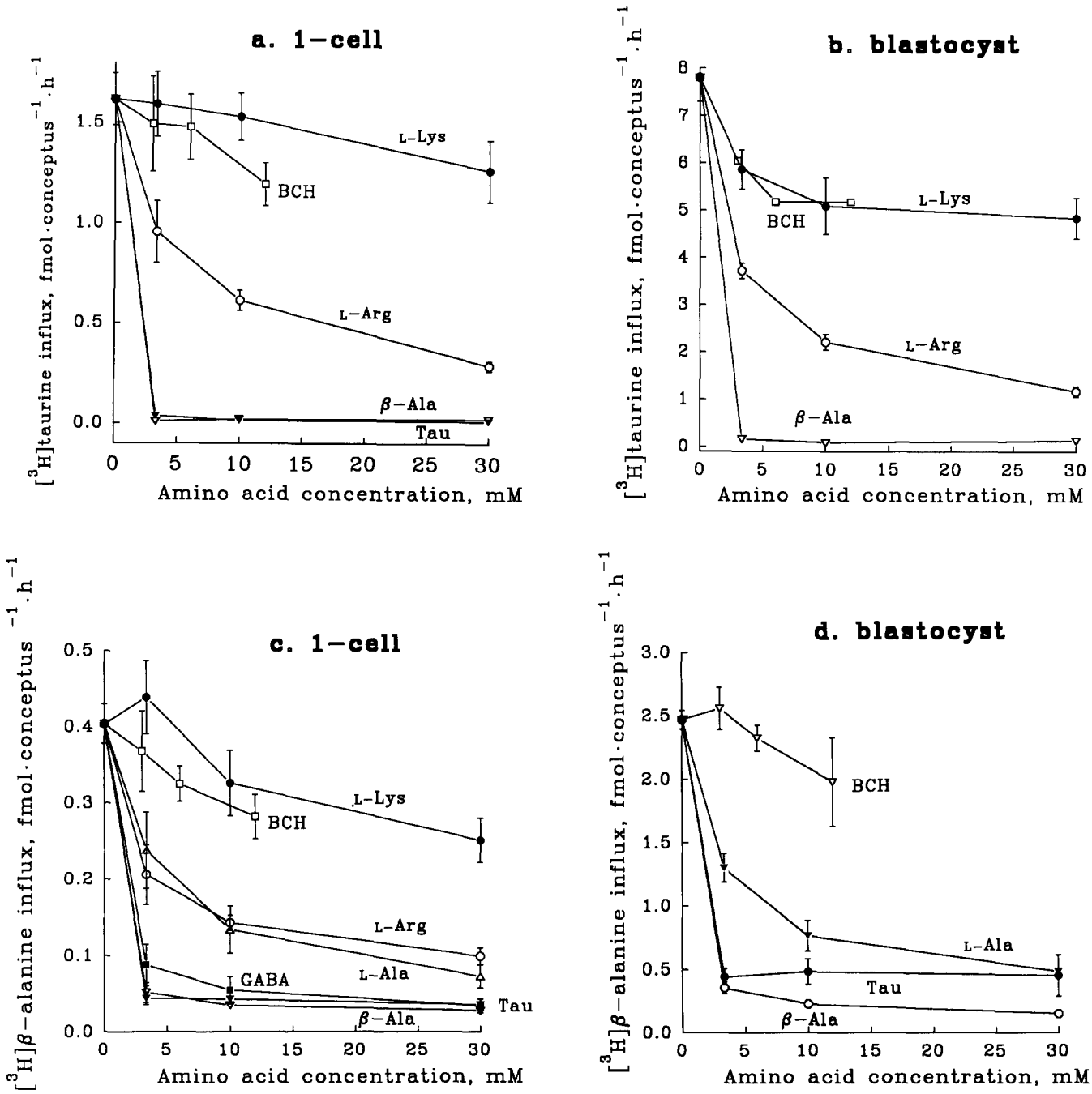

Fig. 2. Inhibition of Na^+-dependent taurine (a and b) and β-alanine (c and d) uptake by different concentrations of various amino acids. One-cell conceptuses (a and c) or intact blastocysts (b and d) were incubated with 1.8 μM [^{3}H]taurine (28 Ci/mmol) or 0.5 μM β-[^{3}H]alanine (93 Ci/mmol) for 15–60 min in MFM. A lower concentration of [^{3}H]taurine was used to produce data in this figure vs. Fig. 1 in order to hold the concentration of radiolabel constant. Since both 1.8 and 2.9 μM taurine are well below the K_m values for taurine transport (e.g., K_m = 14 μM for Na^+-dependent taurine uptake, Fig. 4) both of these taurine concentrations would yield virtually identical results in these experiments (also see Materials and methods). Each point represents the mean ± S.E. uptake of 4–15 replicate determinations (3–5 conceptuses/determination) obtained in 2–7 independent experiments (two determinations and one experiment for effect of BCH on taurine uptake in blastocysts). Uptake was not influenced significantly by 30 mM mannitol, so these data were combined with control data to produce the mean ± S.E. at zero mM. BCH, 2-amino*endo*bicyclo[2.2.1]hexane-2-carboxylic acid; GABA, γ-aminobutyrate; Tau, taurine.

Table 2
Effect of various amino acids on taurine-resistant Na^+-dependent β-[^{3}H]alanine uptake by blastocysts

Amino acid (10 mM)	Taurine-insensitive β-alanine uptake (% of control)
Control	100±2
BCH	34±4 **
L-Alanine	27±3 **
Taurine (total 20 mM)	76±7
β-Alanine	41±2 **
L-Lysine	30±2 **
L-Aspartate	82±5
L-Leucine	27±2 **
γ-Aminobutyrate	63±4 *

Experiments were performed as described in the legend of Fig. 2 except that 10 mM taurine was also present under all conditions. Statistically significant inhibition is indicated by single ($P < 0.05$) or double ($P < 0.01$) asterisks. BCH, 2-amino*endo*bicyclo[2.2.1]hexane-2-carboxylic acid.

pendent taurine transport increased in 1-cell conceptuses when the osmolarity of the medium was raised, whereas the reverse occurred in blastocysts (Fig. 5).

Stimulation of Na^+-independent transport of β-amino acids by hypoosmotic stress

Na^+-independent uptake of taurine and β-alanine increased 2- to 10-fold in preimplantation conceptuses at all stages of development when Na^+-free media were diluted in half with water (Figs. 1b, 5 and 6; some data are not shown). In contrast, Na^+-independent L-alanine uptake by 1-cell conceptuses did not increase (Fig. 6) or increased only slightly (Fig. 1b) in hypotonic media. In addition, Na^+-independent L-valine uptake by intact or collapsed blastocysts was lower in hypotonic medium than in more nearly isotonic medium (not shown). The greater Na^+-independent taurine uptake disappeared when 1-cell conceptuses were returned to normal medium or recovered their initial volumes in hypotonic medium (Fig. 6). Nevertheless, the ten-fold increase in Na^+-independent taurine uptake by 1-cell conceptuses transferred to choline-containing hypotonic medium (Fig. 6) was several-fold greater than the associated two-fold increase in their cell volume (note that the axis indicating volume in Fig. 6 does not begin at zero). Hence, the increase in taurine uptake could not be accounted for solely by a greater cell volume into which taurine could be transported. Na^+-independent taurine uptake also increased in 1-cell conceptuses when choline Cl was replaced by twice as much urea as the major osmolite in nearly isotonic medium presumably because urea

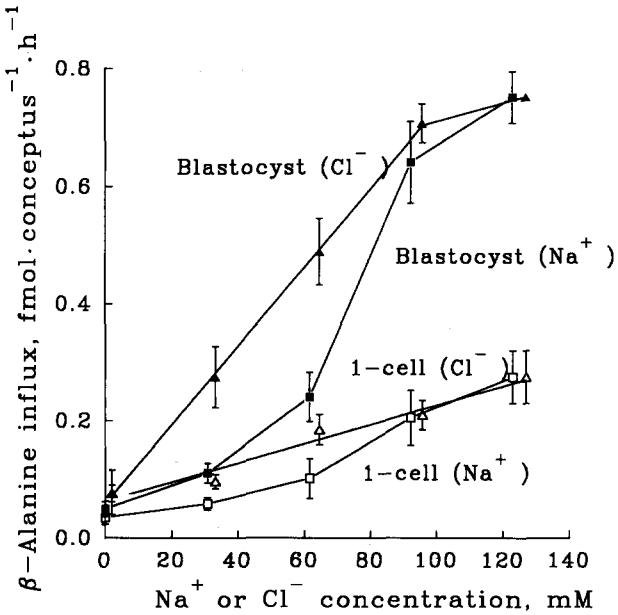

Fig. 3. Effect of the Na^+ or Cl^- concentration on uptake of β-alanine by 1-cell conceptuses and intact blastocysts. Conceptuses were incubated with 0.5 μM β-[^{3}H]alanine (93 Ci/mmol) for 30 (blastocysts) or 60 (1-cell conceptuses) min in MFM or this medium in which some or all of the Na^+ or Cl^- was replaced with K^+ or $MeSO_4^-$, respectively. 10 mM L-lysine was also present to inhibit uptake via the taurine resistant component of β-alanine transport which we attribute to system $B^{o,+}$ [17]. Each point represents the mean±S.E. uptake of 6 or 7 replicate determinations (3–5 conceptuses/determination) obtained in three independent experiments.

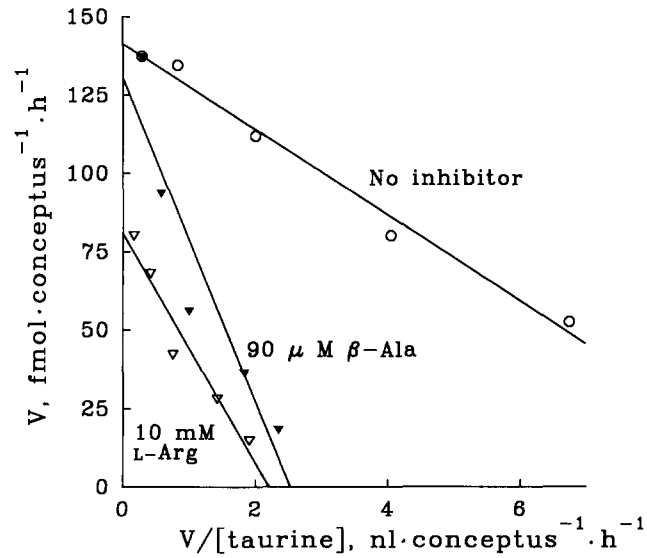

Fig. 4. Competitive inhibition of Na^+-dependent taurine uptake by β-alanine. To generate Hofstee plots, intact blastocysts were incubated with the indicated concentrations of taurine (7.8–488 μM; 1.8 μM [^{3}H]taurine; 28 Ci/mmol) for 60 min in MFM. Nonsaturable uptake (estimated by measuring radiolabeled [^{3}H]taurine uptake in the presence of 10 mM nonradioactive taurine) was subtracted from total uptake to produce the data presented. For each point, the mean uptake was calculated from five replicate determinations (3–5 blastocysts/determination) obtained in two independent experiments. The K_m and V_{max} values were calculated from the slope and y intercept of the regression line in the absence of β-alanine or L-arginine to be 14 μM and 141 fmol (blastocyst)$^{-1}$ h^{-1}. The K_i value for β-alanine was calculated from the formula, K_i = [inhibitor]/((apparent K_m/K_m)−1), to be 32 μM.

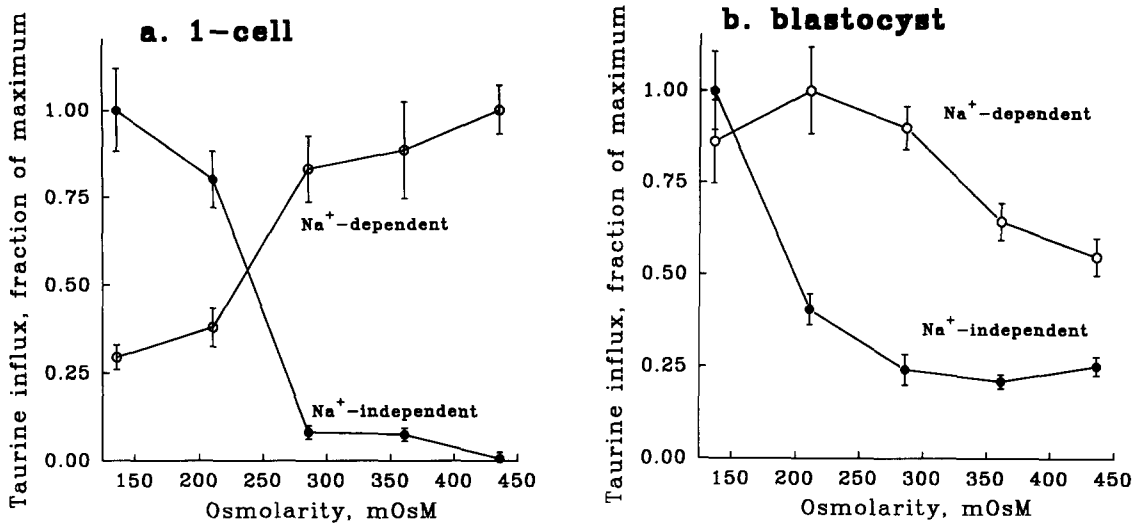

Fig. 5. Effect of osmolarity on Na^+-dependent and Na^+-independent taurine uptake by 1-cell conceptuses (a) and blastocysts (b). Conceptuses were incubated with 1.8 μM [^{3}H]taurine (28 Ci/mmol) for 30 (blastocysts) or 60 (1-cell conceptuses) min in MFM or this medium in which Na^+ was replaced with K^+. The MFM or Na^+-free MFM was diluted in half with water after which the osmolarity was adjusted to the indicated values with sucrose. Na^+-dependent uptake was calculated by subtracting Na^+-independent uptake from total uptake in MFM, although the same conclusions applied regarding the effect of osmolarity on Na^+-dependent uptake even if total uptake was assumed to represent Na^+-dependent uptake. Similar results were obtained for Na^+-dependent β-alanine uptake by blastocysts even after they were collapsed (not shown). Each point represents the mean ± S.E. uptake of 6–8 replicate determinations (4–5 conceptuses/determination) obtained in two independent experiments. Na^+-dependent uptake by blastocysts and Na^+-independent uptake by 1-cell conceptuses and blastocysts was more rapid in hypotonic than in hypertonic media ($P < 0.01$ in each case), whereas the reverse was true for Na^+-dependent uptake by 1-cell conceptuses ($P < 0.01$).

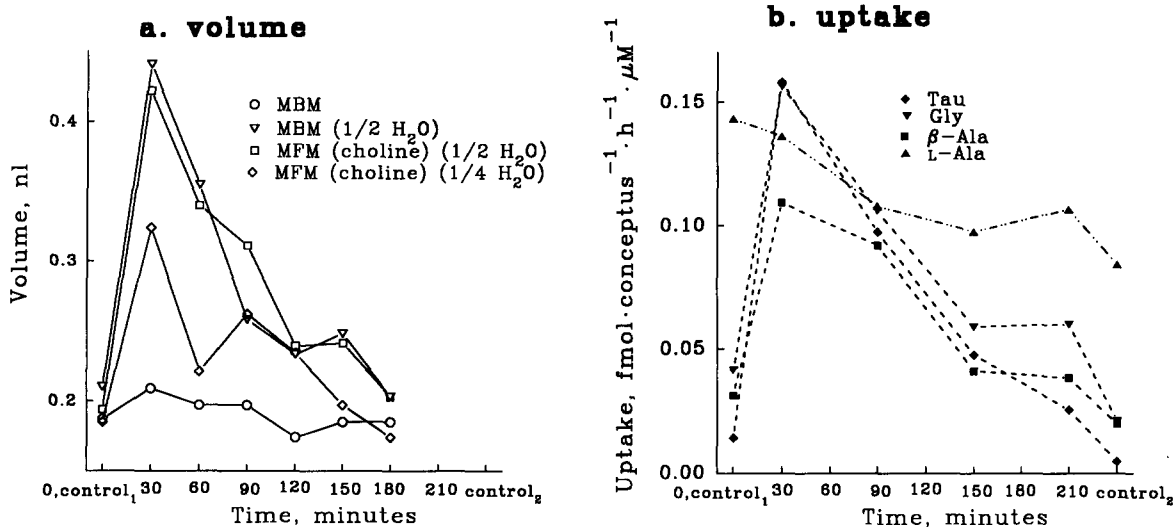

Fig. 6. Effect of time in hypotonic medium on the volume of 1-cell conceptuses (a) and their hypotonically stimulated Na^+-independent ability to take up taurine, β-alanine, glycine and L-alanine (b). Conceptuses were incubated in MBM, Na^+-free MFM (choline substituted for Na^+) or one of these media diluted in half or by 1/4 with water. After the indicated times, the diameters of conceptuses were measured with a micrometer (a) or they were incubated with 1.8 μM [^{3}H]taurine, [^{3}H]glycine or L-[^{3}H]alanine or 0.8 μM β-[^{3}H]alanine for 60 min in Na^+-free MFM diluted in half with water (b). Since uptake was measured over 60 min, the uptake that was detected was plotted to represent uptake 30 min into the labeling period (i.e., uptake after an additional 30 min in hypotonic medium). Control uptake was measured in Na^+-free, MFM prior to ($control_1$) or 180 min after ($control_2$) incubation in Na^+-free MFM diluted in half with water. Also plotted at $control_1$ is the volume of 1-cell conceptuses just prior to exposure to hypotonic medium. Each point represents the mean volume of 8–28 1-cell conceptuses (calculated from the formula $V = (4/3)\ \pi r^3$) or the mean uptake ±S.E. of 4–9 replicate determinations (4–7 conceptuses/determination) obtained in 2–5 independent experiments. In the case of cell size, statistical analyses were performed on the actual measurements that were made (i.e., cell diameters) prior to using these measurements to calculate volume. Both volume ($P < 0.01$) and hypotonically stimulated taurine ($P < 0.01$) and glycine ($P < 0.05$) transport were significantly reduced below their maximum within 90 min after the onset of incubation of 1-cell conceptuses in Na^+-free MFM diluted in half with water. Such was also the case for 1-cell volume in hypotonic MBM ($P < 0.01$) and taurine uptake in blastocysts ($P < 0.05$, not shown). Na^+-independent β-alanine uptake by 1-cell conceptuses also varied in association with the volume of these cells ($P < 0.01$). In contrast, Na^+-independent L-alanine uptake was not influenced by hypotonic medium in a statistically significant manner.

freely permeates and thus also swells these cells (data not shown). Na^+-independent β-alanine and glycine (but not L-alanine) uptake also correlated with cell volume changes in 1-cell conceptuses (Fig 6). Moreover, the initial increase in hypotonically stimulated Na^+-independent taurine uptake by blastocysts was followed by a decrease in uptake (data not shown), although the possible associated volume changes in trophoblast cells could not be as easily and directly measured as in 1-cell conceptuses.

Although the extremely hypotonic conditions used in many of these studies undoubtedly produce some cellular abnormalities, the volume regulation observed in 1-cell conceptuses (Fig. 6) probably cannot be attributed to swelling of the cells followed by their death. First, Na^+-dependent transport, which presumably requires maintenance of membrane integrity, was not lost in 1-cell conceptuses or blastocysts in hypotonic medium (Fig. 5). In fact, this Na^+-dependent β-alanine transport returned to normal levels (i.e., 116 ± 19% of control) in 1-cell conceptuses that were returned to isotonic, Na^+-containing medium after incubation of the conceptuses for 90 min in the Na^+-free, hypotonic medium used for most of the studies reported in Fig. 6. Furthermore, the considerable changes in the ability of 1-cell conceptuses to take up taurine, β-alanine and glycine in hypotonic, Na^+-free medium occurred under conditions in which L-alanine transport activity remained relatively high and did not change in a statistically significant manner (Fig. 6). Finally, the hypotonically stimulated transport associated with volume changes in conceptuses was susceptible to inhibition by a variety of substances that are unlikely to influence transport across a membrane that has simply lost its integrity (see below). One of these inhibitors, 1.0 mM 4,4′-diisothiocyanostilbene-2,2′-disulfonate (DIDS), slowed but did not prevent a regulatory volume decrease by 1-cell conceptuses in hypotonic medium (data not shown).

The Na^+-independent taurine uptake, that was stimulated by hypotonic medium, was inhibited by niflumate, *N*-ethylmaleimide (NEM), and NaN_3 (10 mM) but not by furosemide, iodoacetate, KCN (2 mM), ouabain (4 mM) or any of nine amino acids tested (Tables 3 and 4 and data not shown). In addition, DIDS inhibited hypotonically stimulated taurine uptake in 1-cell conceptuses but not in blastocysts, and niflumate was a considerably stronger inhibitor in 1-cell conceptuses than in blastocysts (Table 3). In contrast, Na^+-independent L-valine transport via system L in conceptuses was not inhibited by DIDS, NEM or NaN_3 (data not shown).

Table 4
Effect of various amino acids on hypotonically stimulated Na^+-independent [^{3}H]taurine uptake by 1-cell conceptuses and blastocysts

Amino acid (10 mM)	[^{3}H]Taurine uptake (% of control)		
	1-cell	blastocyst	collapsed blastocyst
Control	100± 5	100± 9	100±13
Taurine	76±11	111±11	127±10
β-Alanine	72± 7	99±16	133±12
Glycine	82±12	136±15	117±10
L-Proline	82±12	119±18	102±18
γ-Aminobutyrate	83±10	149±27	98±12
L-Alanine	75±12	119± 9	88±18
L-Leucine	69± 8	154±18	118±10
L-Aspartate	73± 7	139±42	92±13
L-Arginine	90± 8	138±20	125±22

[^{3}H]Taurine uptake was measured as described in footnote 1 of Table 3. Control groups containing 5 mM KCl, 10 mM KCl or 10 mM sucrose were indistinguishable and so were combined. No statistically significant effects of nonradioactive amino acids on [^{3}H]taurine uptake were detected.

Table 3
Effect of various substances on hypotonically stimulated Na^+-independent taurine uptake by 1-cell conceptuses and blastocysts

Substance	Mean ± S.E. uptake (% of control) [a]	
	1-cell	blastocyst
DIDS		
1.0 mM	26± 2**	86±12
0.1 mM	79±10	89± 7
0.01 mM	111± 9	112± 9
Niflumate		
1.0 mM	9± 2**	79± 5*
0.3 mM	58± 5**	–
0.1 mM	112± 4	108±11
Furosemide		
2.0 mM	84±14	–
1.0 mM	85± 5	101± 8
0.2 mM	101± 7	130±29
Iodoacetate		
1.0 mM	94±12	90±17
0.5 mM	107±12	92± 8
N-ethylmaleimide		
2.0 mM	29±11**	60± 4**
0.7 mM	32±12**	73± 9
0.2 mM	41± 5**	84± 7

Taurine uptake was measured as described in the legend of Fig. 6 immediately after transferring conceptuses to Na^+-free MFM diluted in half with water except that in some cases a total of up to 20 replicate determinations were made (e.g., effect of 1.0 mM furosemide). Statistically significant inhibition is indicated with single ($P < 0.05$) or double ($P < 0.01$) asterisks. Hypotonically activated glycine uptake was inhibited in a similar manner (data not shown). In addition, except for DIDS, the effects of the substances on blastocysts were about the same after they had been collapsed (data not shown.)

[a] Inhibition to about 10% in 1-cell conceptuses and 25% in blastocysts was considered complete since uptake increased about ten- and four-fold, respectively, when the medium was diluted in half with water (Fig. 6 and data not shown for blastocysts).

4. Discussion

Hypotonic stress stimulates Na^+-independent taurine transport via processes in conceptuses that appear to be different from the Cl^- channel or the anion exchanger

Substances that characteristically inhibit the anion exchanger and the Cl^- channel have been used to produce data supporting the hypothesis that hypotonically stimulated taurine transport occurs via one or the other of these processes in some vertebrate cells [10–12]. In the present study, the weak or lack of inhibition of taurine uptake by some of these substances supports the proposition that hypotonically stimulated taurine transport does not occur via either of these processes in preimplantation conceptuses. 100 μM DIDS, niflumate and furosemide appear to inhibit taurine transport via the anion exchanger or Cl^- channel [10,11], but considerably more niflumate is needed to inhibit hypotonically activated Na^+-independent taurine transport in conceptuses, and even 2.0 mM furosemide is ineffective in this regard (Table 3). Moreover, 1.0 mM DIDS does not inhibit hypotonically activated Na^+-independent taurine transport in blastocysts, although it does inhibit transport in 1-cell conceptuses. Hence, inhibition by DIDS distinguishes the hypotonically activated Na^+-independent taurine transport process in 1-cell conceptuses from the one in blastocysts as does the relative strength of inhibition by niflumate (Table 3). It should be interesting to learn whether and by what mechanisms these different hypotonically activated Na^+-independent transport processes contribute to development of preimplantation conceptuses (see further discussion at the end of this section). Regardless of their functions, however, these apparently novel transport processes distinguish early mouse conceptuses from other cells that have been studied, as is also the case for numerous other novel transport processes in preimplantation conceptuses (e.g., [15,17,28–31]).

Nature of the Na^+-dependent transport of β-amino acids in conceptuses

Nearly complete and relatively strong (Fig. 2, Table 1) inhibition of Na^+-dependent taurine and β-alanine transport by each other and by GABA but not by α-amino acids is consistent with the conclusion that system β is present in 1-cell conceptuses and blastocysts. The nonlinear relationship between taurine-sensitive β-alanine uptake and the Na^+ concentration and the linear relationship between uptake and the Cl^- concentration (Fig. 3) are also consistent with the conclusion that system β is present in conceptuses. β-Alanine competitively inhibits taurine transport in blastocysts, while the kinetics of L-arginine inhibition appear to be more complex (Fig. 4). Arginine is also a much weaker inhibitor of taurine transport than is β-alanine (Fig. 4). Arginine may be able to interact weakly with system β via its guanidino rather than its α-amino group. By these criteria, taurine and β-alanine compete for transport via system β.

System β probably is present in conceptuses throughout the preimplantation period (Fig. 1) although inhibition analyses were not performed at every stage of development. Nevertheless, mRNA extracted from conceptuses at several stages of preimplantation development were used with reverse transcription and the polymerase chain reaction (RT/PCR) to amplify DNA (Fig. 7) apparently corresponding in sequence to a portion of the cDNA encoding the taurine transporter (TAUT) in mouse brain [32]. Hence, the same Na^+-dependent system probably transports β-amino acids at each of these preimplantation stages. Moreover, these data support the theory that the TAUT protein is a major component of transport system β in early conceptuses (see Ref. 15 for further discussion of possible distinctions between transport proteins (transporters) and transport systems).

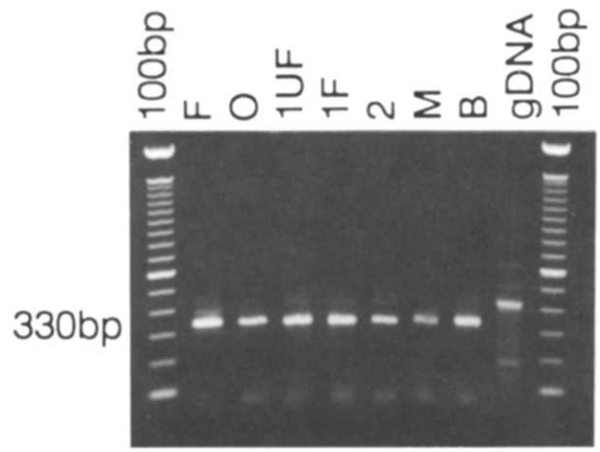

Fig. 7. Use of reverse transcription and the polymerase chain reaction to produce and amplify DNA corresponding to a segment of taurine transporter (TAUT)-mRNA that was isolated from mouse eggs and preimplantation conceptuses. Reverse transcription and the polymerase chain reaction were performed as described by Rappolee and associates [39]. Primers 21 nucleotides long were selected to correspond to known segments of the cDNA encoding the TAUT transport protein in mouse brain [32]. The primers were (5'-primer) 5'-TCTTCAGAGGAGCATCTCAAG-3', 5' = residue 198 and (3'-primer) 5'-GACCTCCAAGAAAAACACAGG-3', 3' = residue 507. Hence, primers were expected to produce a DNA fragment of 330 bp. mRNA from 5–10 ovarian follicles, oocytes or conceptuses and 55 PCR cycles were used to produce the ethidium bromide-stained DNA shown. Abbreviations: 100 bp, 100 base pair ladder; F, ovarian follicles; O, immature oocytes; 1UF, unfertilized eggs; 1 F, fertilized eggs; 2, 2-cell conceptuses; M, morulae; B, blastocysts; gDNA, genomic DNA isolated from mouse liver (PCR amplification of about 0.9 μg of genomic DNA is shown). A partial sequence (125 bp) of the DNA amplified from oocyte mRNA corresponded exactly to the known sequence of a portion of the mouse brain TAUT cDNA. The nucleotide sequence was determined using an Amplitaq cycle sequencing kit (Perkin-Elmer).

In addition to system β, a component of Na^+-dependent β-alanine transport in blastocysts is relatively insensitive to inhibition by taurine. The latter less conspicuous component of β-alanine transport is inhibited by a broad scope of cationic and zwitterionic but not anionic α-amino acids (Table 2). By these criteria, we attribute this component of β-alanine transport to system $B^{o,+}$. We found previously that β-alanine weakly inhibited L-alanine uptake via system $B^{o,+}$ [17]. The near rejection of taurine by system $B^{o,+}$ apparently reflects reduced reactivity of the system not only with the β-configuration but also with sulfonyl vs. carboxyl groups.

Regulation of transport via system β and the Na^+-independent hypotonically stimulated transport processes

In another study, Na^+-independent taurine transport was found to increase, whereas Na^+-dependent transport via system β decreased when fish red blood cells were exposed to hypotonic medium [9]. The latter decrease could not be fully accounted for by the lower Na^+ and Cl^- concentrations in hypotonic medium. In skate hepatocytes, transport by system β apparently is not influenced by hypotonic medium per se [14]. In 1-cell conceptuses, transport of 1.8 μM taurine by system β is lower in hypotonic than in hypertonic medium, whereas the reverse is true for blastocysts (Fig. 5). These changes in transport via system β in conceptuses are due to differences in medium osmolarity rather than ion concentrations since the ion concentrations were held constant and the osmolarity was varied using sucrose (Fig. 5). Hence, transport via system β appears to be influenced by osmolarity differently in different types of cells, perhaps because system β has different functions in these cells (see below and Fig. 8).

In contrast to system β transport in 1-cell conceptuses, hypotonically stimulated Na^+-independent taurine transport is, of course, higher in hypotonic than in hypertonic medium (Fig. 5). The hypotonically stimulated Na^+-independent increase in taurine transport returns to normal when 1-cell conceptuses recover their initial volumes in hypotonic medium (Fig. 6). Hence, a decrease in the environmental osmolarity results in a temporary increase in cell volume followed by a regulatory volume decrease (or, perhaps more correctly, a regulatory increase in intracellular protein concentration [33]) that could be associated with both a loss of taurine via the hypotonically stimulated Na^+-independent pathway and a decreased capacity to take up taurine via system β in 1-cell conceptuses. These changes in taurine transport activities are in the reverse directions for 1-cell conceptuses in hyperosmotic medium (Fig. 5) and so could conceivably contribute to a regulatory volume increase (and decrease in intracellular protein concentration) in this case.

In blastocysts, in contrast to 1-cell conceptuses, system β activity is higher in hypotonic than in hypertonic media (Fig. 5). Hence, system β might cause blastocyst cells to swell further in hypotonic conditions. A similar intensification of cell swelling by some Na^+-dependent amino acid transport systems in rat liver cells may contribute to cell volume related changes in metabolism in this organ [34]. For example, increases in cell volume in hypotonic medium, or as a result of insulin stimulation, are exacerbated by concomitant increases in Na^+-dependent amino acid uptake. These increases in cell volume appear to result in greater protein synthesis and accumulation [33,34]. In this regard, preimplantation mouse conceptuses begin to increase their protein content only after they reach the blastocyst stage of development (summarized in Ref. 28). For these reasons, an increase in Na^+-dependent taurine uptake in hypotonic medium might reflect a normal aspect of metabolic regulation in blastocysts where protein synthesis and accumulation can keep pace with multiple causes for an increase intracellular water. Presumably, 1-cell conceptuses cannot accumulate protein through greater synthesis than degradation and so decrease system β activity in hypotonic medium (Fig. 5). In this regard, it has been proposed that cells defend against changes in their intracellular protein concentrations rather than changes in their volumes [33]. Fig. 8 depicts a model by which increased taurine uptake via system β could help to favor anabolic processes in blastocysts, although further studies are needed to test this hypothesis.

Relationship of taurine transport regulation to development of conceptuses in hypo- and hyperosmotic media

Taurine (1–20 mM) increases the frequency at which 2-cell conceptuses develop into blastocysts in slightly hypotonic media (i.e., 280–285 mosmol/kg) [2]. In contrast, taurine (0.1–32 mM) does not increase the frequency of development of 2-cell conceptuses into blastocysts in oviductal fluid-like medium [16]. The latter medium is based on the ion composition of mouse oviductal fluid, and it is hypertonic (370 mosmolar). We suggest that taurine in slightly hypotonic media helps to maintain intracellular taurine concentrations at the relatively high levels of this amino acid normally found in preimplantation conceptuses [3]. In this regard, we have found that the quantity of taurine in blastocysts that develop in slightly hypotonic medium without added taurine (0.56 ± 0.12 pmol/conceptus) is considerably lower than in blastocysts that develop in vivo (2.28 ± 0.42 pmol/conceptus) (Dickinson and Van Winkle, unpublished results). Conceptuses might lose taurine via their hypotonically stimulated Na^+-independent transport process in slightly hypotonic medium that does not contain added taurine (Fig. 8a), whereas this transport activity might be relatively slow in vivo or

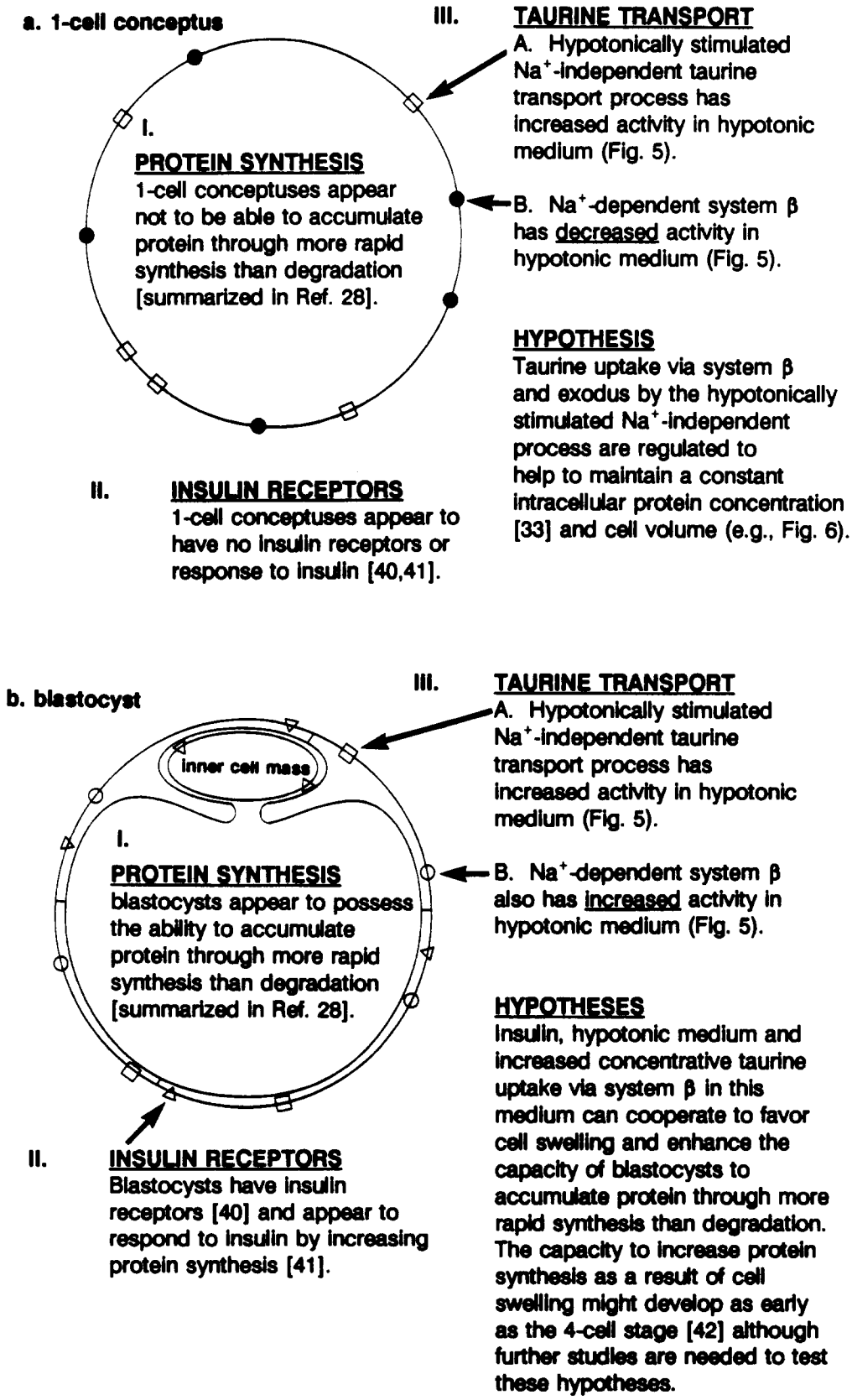

Fig. 8. Proposed model to explain different regulation of Na^+-dependent taurine transport activity (system β) in 1-cell conceptuses (a) vs. blastocysts (b) in hypotonic and hypertonic media (Fig. 5). Filled circles, hypotonically *inhibited* system β in 1-cell conceptuses; open circles, hypotonically *stimulated* system β in blastocysts; squares, hypotonically stimulated, Na^+-independent taurine transport processes in both types of conceptuses; triangles, insulin receptors in blastocysts.

in hypertonic medium in vitro. Presumably, our assay was not sensitive enough to detect the relatively small difference in hypotonically activatable Na^+-independent taurine transport that may exist between conceptuses in slightly hypotonic vs. hypertonic media (Fig. 5). Nevertheless, a possibly continuous and relatively small need to oppose a tendency of cells to swell (perhaps in order to maintain a constant intracellular protein concentration) over several days of culture in slightly hypotonic medium could conceivably deplete taurine stores. In this regard, taurine levels in conceptuses remain relatively high during preimplantation development when taurine is also present in slightly hypotonic medium (our unpublished data). Hence, exogenously supplied taurine could help to maintain relatively high levels of taurine and greater viability [2] in conceptuses in slightly hypotonic medium, whereas exogenously supplied taurine might not be needed for this purpose in conceptuses in hypertonic medium [16]. The presence of exogenously supplied taurine in slightly hypotonic medium would oppose but should not prevent the regulatory volume decrease of cells in this medium since inhibition of hypotonically stimulated Na^+-independent taurine transport with 1.0 mM DIDS slowed but did not prevent recovery of their initial volumes by 1-cell conceptuses in hypotonic medium (data not shown). Presumably, conceptuses have mechanisms in addition to taurine transport to achieve volume (or protein concentration) regulation, as is the case for other cells (e.g., [33,34]).

If the preceding hypothesis is correct, then taurine may have functions in the cells of early conceptuses in addition to helping the cells achieve a regulatory volume decrease by releasing taurine in environments that cause cells to swell. These additional functions appear to require taurine to be present in cells, and they are likely to relate to the general ability of taurine to help cells respond to externally applied stresses [1]. The mechanisms by which taurine performs such functions are still under investigation, although in some cases the mechanisms probably involve taurine as an antioxidant, a modulator of Ca^{2+} action and a stimulator of Cl^- fluxes [1]. It has been suggested [35] that taurine could influence development of preimplantation embryos by inhibiting Na^+/K^+-ATPase, although the effect of taurine on Na^+/K^+-ATPase activity in conceptuses [36] has not been measured.

Taurine transport and metabolism also need to be studied under other circumstances that may influence them in preimplantation conceptuses. For example, both glutamine and insulin improve development of conceptuses in vitro [37,38], and both of these substances appear to exert their anabolic effects on hepatocytes at least in part by causing the cells to swell [34]. The effects of insulin and glutamine on taurine transport in conceptuses have not, however, been determined. We are currently investigating the effects of insulin on the activities of numerous transport processes that may be involved in volume (or intracellular protein concentration) regulation including numerous amino acid transport systems [15,28] and a novel K^+ transport system in preimplantation mouse conceptuses [29].

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