Biochimica et Biophysica Acta, 1146 (1993) 38–44

BBAMEM 75896

The cation receptor subsite of the choline transporter in preimplantation mouse conceptuses resembles a cation receptor subsite of several amino acid transporters

Lon J. Van Winkle, Allan L. Campione, David F. Mann and Howard G. Wasserlauf

Department of Biochemistry, Chicago College of Osteopathic Medicine, Downers Grove, IL (USA)

(Received 5 August 1992)

Key words: Membrane transport; Choline transport; Amino acid transport; Preimplantation conceptus; (Preimplantation embryo)

Mediated choline transport in preimplantation mouse conceptuses was inhibited competitively by Na^+ and other cationic osmolites. Uptake of choline by conceptuses was also inhibited relatively strongly by ethanolamine, hemicholinium-3, harmaline, harmalol and harmine. The K_i values for inhibition of choline transport by most of the latter inhibitors were of the same order of magnitude as the K_m value for choline transport (~ 100 μM). To our knowledge, we are the first to show that mediated 'Na^+-independent' choline transport is, nevertheless, inhibited strongly by the Na^+-site inhibitor, harmaline. Inhibitions by harmaline, Na^+ and other cations have been used to draw a parallel between the substrate receptor sites of amino acid transport systems y^+ and $b^{o,+}$. We suggest that the latter parallel should be extended to include the Na^+-independent mammalian choline transporter. In addition, the choline transport activity in conceptuses increased by more than 100-fold between the 2-cell and blastocyst stages of development. Mouse blastocysts probably utilize choline for the synthesis of membrane phospholipids during cellular differentiation and when they begin to grow about ten hours prior to implantation. Since we show here that mouse conceptuses develop the capacity to transport choline prior to the onset of growth, some of the choline utilized for growth could come from an exogenous source.

Introduction

Mammalian cell membranes contain both Na^+-dependent (i.e., Na^+-stimulated) and Na^+-independent (not Na^+stimulated) choline transport systems. Nevertheless, some of the other characteristics of these two categories of choline transporters are similar, and they resemble some of the characteristics of amino acid transport systems. In general, Na^+-dependent choline transport systems have lower K_m values for choline than do Na^+-independent systems although exceptions to this generalization are known. For example, the K_m value for Na^+-independent choline transport by glial cells at low ionic strength is lower than the K_m value for Na^+-dependent transport by neuronal cells at physiological Na^+ concentrations [1]. In addition, both the Na^+-dependent and Na^+-independent choline transporters are inhibited by choline analogs, such as hemicholinium-3 (HC-3) (e.g., Refs. 1–11), and the Na^+-dependent system in rat striated synaptosomes is inhibited by harmala alkaloids [8,12]. The effects of harmala alkaloids on Na^+-independent choline transport apparently have not been investigated perhaps because these alkaloids, such as harmaline, are known to inhibit Na^+ binding [13–17]. Nevertheless, the Na^+-independent amino acid transport systems asc, $b^{o,+}$ and y^+ are inhibited by harmaline [17–19], and systems $b^{o,+}$ and y^+ are inhibited by Na^+ and other cations [18,19]. Similarly, the 'Na^+-independent' choline transporter in glial cells [1], red blood cells [20] and placenta [6] appears to be inhibited by Na^+. Thus, it is possible that the 'Na^+-independent' choline transporter is also inhibited by harmala alkaloids.

These characteristics of amino acid transporters have been used to help to draw a parallel among the structures of their substrate receptors sites [17–19]. We propose that these characteristics might also be used to extend this parallel to the Na^+-inhibited and perhaps to the Na^+-stimulated choline transporters. In this regard, the amino acid sequence of the Na^+-independent yeast choline transporter has significant similarities to yeast amino acid transporters [21], and its transmembrane topology is also homologous to that of the mammalian amino acid transport system y^+ [22]. Because of the possible structural similarities among

Correspondence to: L.J. Van Winkle, Department of Biochemistry, Chicago College of Osteopathic Medicine, 555 31st Street, Downers Grove, IL 60515, USA.

mammalian choline and amino acid transporters, and because exogenous choline might be taken up and utilized for metabolic processes, such as incorporation into the phospholipids of cell membranes, we characterized choline transport in preimplantation mouse conceptuses.

Materials and Methods

Conceptuses were obtained as described previously [23–25] from sexually mature 8–11-week-old ICR mice (Harlan Sprague Dawley, Inc.). Mice were induced to ovulate and mate utilizing gonadotropins [26]. 1- and 2-cell conceptuses were obtained from oviducts about 19 and 43 h, respectively, after administration of human chorionic gonadotropin. Blastocysts were obtained from the uterus approx. 94 h after injection of this hormone. Conceptuses were washed and stored for 6 h or less in Brinster's medium [27] in a humidified atmosphere of 5% CO_2 in air at 37°C (pH 7.4). Choline transport activity was not observed to change in conceptuses during incubation in Brinster's medium for 6 h.

Conceptuses were incubated with [^{3}H]choline (75 Ci/mmol) (Amersham) and the indicated nonradioactive substances in a 15 mM 4-morpholine propanesulphonic acid (Mops)-buffered solution of either 288 mM sucrose, 288 mM trimethylamine *N*-oxide, 144 mM NaCl, 144 mM LiCl or 144 mM KCl (pH 7.2) at 37°C. These solutions were selected for use in these studies because they have been used to assess the Na^+-dependence of choline uptake by human fibroblasts [4]. Choline transport was frequently studied at concentrations near 1 μM in order to help insure that if a transporter with a K_m value near 1 μM were present, it would not be obscured by a transporter with relatively high K_m and V_{max} values [18,24,28–30]. The choline transport we detected could not be attributed instead to choline binding since virtually no binding of [^{3}H]choline to conceptuses was detected after conceptuses had been killed by freeze thawing them inside their zona pellucidae. (This treatment reduced the counts per min (cpm) associated with blastocytes to $0.3 \pm 0.1\%$ of the cpm associated with live blastocysts exposed to 0.67 μM [^{3}H]choline in either sucrose- or NaCl-based medium.) Incubations were short enough to estimate initial velocities of uptake (i.e., 5 min or less), and the extracellular concentrations of choline and other substances remained constant during the course of experiments as discussed previously [30,31].

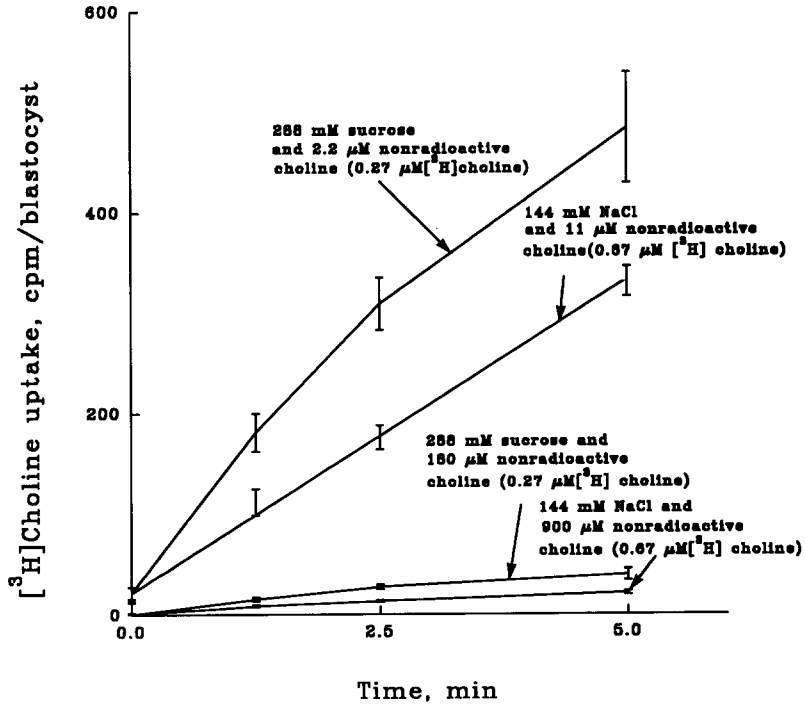

Fig. 1. [^{3}H]Choline uptake by mouse blastocysts increased nearly linearly with time for 2.5–5.0 min at total choline concentrations above 2 μM. Blastocysts were incubated with 0.27 (288 mM sucrose) or 0.67 (144 mM NaCl) μM [^{3}H]choline (75 Ci/mmol) and the indicated concentrations of nonradioactive choline for 0–5 min at 37°C. Each point represents the mean ± S.E. [^{3}H]choline uptake of 7–8 replicate determinations (one (sucrose) or three (NaCl) blastocysts/determination) obtained in three independent experiments. Uptake increased nearly linearly with time for 5 min in most cases (correlation coefficients ≥ 0.99, $P < 0.01$) except for uptake in 288 mM sucrose containing 180 μM nonradioactive choline where uptake increased linearly with time for 2.5 min (correlation coefficient = 0.998 for up to 2.5 min, $P < 0.05$). Uptake was measured to be somewhat above zero at zero time primarily because a few seconds are required to remove conceptuses from the medium containing [^{3}H]choline after they have been placed in the medium. This same small overestimate of measured uptake would occur at each time point so the conclusion that uptake increases nearly linearly with time is still valid.

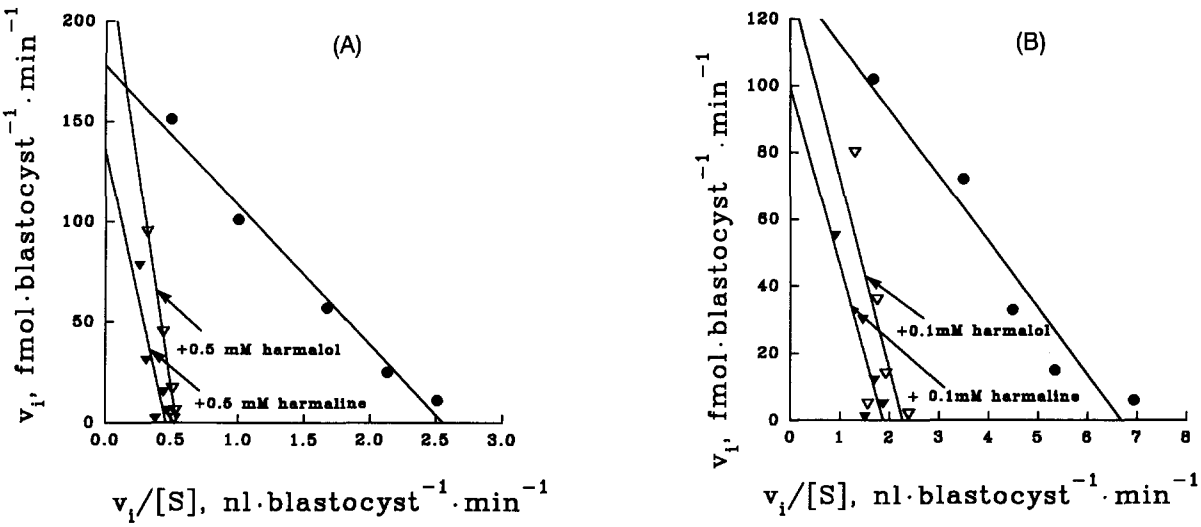

Fig. 2. Competitive inhibition of choline uptake by harmalol and harmaline in 144 mM NaCl (A) or 288 mM sucrose (B). Blastocysts were incubated with the indicated concentrations of choline (0.67 μM [^{3}H]choline), harmalol and harmaline for 1.25 min at 37°C in a Mops-buffered NaCl or sucrose solution (pH 7.2). Nonsaturable uptake was deducted from total uptake by subtracting counts per min (cpm) taken up in the presence of a nearly saturating amount of nonradioactive choline (20 mM in A; 10 mM in B) from cpm taken up at the indicated choline concentrations (Fewer than 6.0% of the cpm had to be subtracted for this correction even at the highest nonsaturating choline concentrations in the figure.) These net cpm were utilized to calculate the velocities of choline uptake at various nonsaturating choline concentrations. Each point represents the mean uptake calculated from 6–8 replicate determinations (usually three blastocysts/determination) obtained in three or four independent experiments. The kinetic parameters that were determined from these data (see Materials and Methods) are included in Table III.

At some choline concentrations above 2 μM, uptake appeared to increase nearly linearly with time for less than 5 min (Fig. 1), so the initial rates of choline uptake were measured over a period of 1.25 min in kinetic studies (e.g., Figs. 2 and 3). After incubation with [^{3}H]choline, conceptuses were processed to determine how much of the substrate they had taken up [23]. A counting efficiency of 41% was utilized to calculate the velocities of uptake from counts per min (cpm) taken up by conceptuses after subtraction of procedural controls. (Cpm in these controls were indistinguishable from background cpm.) In some cases, data are expressed as clearance (velocity/[choline]) in order to facilitate comparison of uptake by blastocysts to uptake by 1- and 2-cell conceptuses at different submicromolar concentrations of [^{3}H]choline. Where appropriate, data were assessed statistically utilizing analysis of variance [32,33]. Values of kinetic parameters were calculated from data in Hofstee plots as follows: $K_m = -\text{slope}$, $V_{max} = y$ intercept, and $K_i = [\text{inhibitor}]/((\text{apparent } K_m/K_m) - 1)$ [34]. All nonradioactive substances and hormones were purchased from Sigma or Behring Diagnostics.

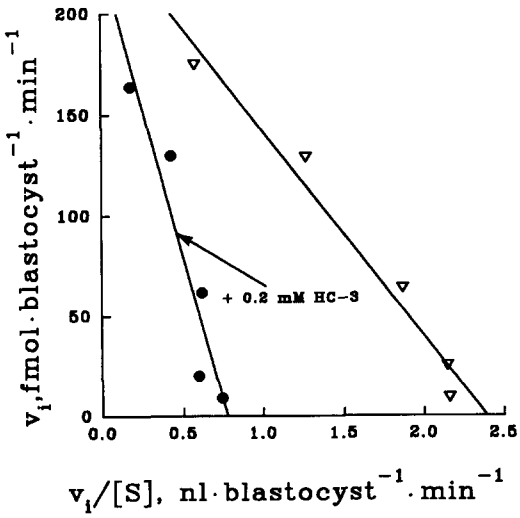

Fig. 3. Competitive inhibition of choline uptake by hemicholinium-3 (HC-3) in 144 mM NaCl. Experiments were performed with blastocysts as described in the legend of Fig. 2.

Results

Choline uptake by preimplantation mouse blastocysts was not stimulated by Na^+ and, in fact, it appeared to be inhibited by this and other cationic osmolites relative to uptake in Mops-buffered sucrose (Table I). Inhibition of choline uptake in solutions of electrolytes relative to uptake in sucrose appeared to be due to cations rather than anions since uptake was influenced more by the nature of the cation at constant [Cl^-] than by the nature of the anion at constant [Li^+] (Table II). In this regard, the Na^+ site inhibitor, harmaline [17–19], strongly inhibited choline transport (Table I). The highly polar but nonionic substance, trimethylamine *N*-oxide (TMAO), also appeared to inhibit choline uptake relative to uptake in sucrose-based medium (Table I), although we have also found repeatedly (Table I and data not shown) that uptake in

TABLE I

Effect of various osmolites, choline analogs and harmala alkaloids on choline uptake by mouse blastocysts

Blastocysts were incubated with 0.27 μM [^{3}H]choline (75 Ci/mmol) and the indicated non-radioactive inhibitor for 5 min at 37°C in Mops-buffered solutions of the indicated osmolite (pH 7.2). The mean uptake ± S.E. was calculated in units of clearance of [^{3}H]choline from the medium (i.e., velocity of uptake/[choline]) utilizing 6–26 replicate determinations (usually three blastocysts/determination) obtained in three to ten independent experiments. Uptake was slower in all treatment groups than in 288 mM sucrose alone ($P < 0.01$), and each substance tested inhibited uptake in isotonic NaCl ($P < 0.01$). In experiments performed separately, uptake in 288 mM TMAO was faster than in 144 mM NaCl ($P < 0.01$), whereas uptake in 144 mM NaCl has not consistently been observed to exceed uptake in 144 mM LiCl. Abbreviations: HC-3, hemicholinium-3; TMAO, trimethylamine *N*-oxide.

Osmolite	Inhibitor	[^{3}H]Choline clearance (nl/blastocyst per min)
Sucrose	none	6.08 ± 0.48
	1.0 mM choline	0.15 ± 0.03
	1.0 mM ethanolamine	1.51 ± 0.14
	1.0 mM harmine	2.05 ± 0.20
	1.0 mM harmalol	2.09 ± 0.22
	1.0 mM harmaline	1.94 ± 0.34
	1.0 mM HC-3	0.49 ± 0.11
TMAO	none	2.25 ± 0.18
LiCl	none	1.12 ± 0.09
NaCl	none	1.74 ± 0.14
	1.0 mM harmine	0.15 ± 0.02
	1.0 mM harmalol	0.27 ± 0.03
	1.0 mM harmaline	0.13 ± 0.02
	1.0 mM HC-3	0.10 ± 0.01

TABLE II

Effect of mono-, di- and trivalent salts of Li^+ and Cl^- on choline uptake by blastocysts

Conceptuses were incubated for 5 min at 37°C with 0.67 μM [^{3}H]choline (75 Ci/mmol) in Mops-buffered sucrose (pH 7.2) or this solution in which an equal osmolar amount of the indicated substance was substituted for part of the sucrose. The mean uptake ± S.E. was calculated from eight or nine replicate determinations (two to three blastocysts/determination) obtained in four independent experiments. Substances that inhibited uptake significantly more than LiCl ($P < 0.01$) are marked with an asterisk (*). The nature of the cation at constant [Cl^-] influenced uptake much more than the nature of the anion at constant [Li^+].

Substance used to partially replace sucrose	% of [^{3}H]choline uptake relative to uptake in sucrose
None (288 mM sucrose)	100 ± 13
Solutions containing 20 mM Cl^-	
20 mM LiCl	86 ± 6
10 mM $MgCl_2$	74 ± 16
6.7 mM spermidine Cl_3	33 ± 3 *
Solutions containing 40 mM Cl^-	
40 mM LiCl	68 ± 4
20 mM $MgCl_2$	44 ± 4 *
13.3 mM spermidine Cl_3	43 ± 2 *
Solutions containing 20 mM Li^+	
20 mM LiCl	86 ± 6
10 mM Li_2SO_4	70 ± 5
6.7 mM Li_3 citrate	72 ± 4
Solutions containing 40 mM Li^+	
40 mM LiCl	68 ± 4
20 mM Li_2SO_4	61 ± 5
13.3 mM Li_3 citrate	49 ± 3 *

TMAO exceeds uptake in NaCl ($P < 0.01$). Choline uptake by blastocysts was also inhibited relatively strongly by ethanolamine, by the choline analogue, hemicholinium-3 (HC-3), and by the harmala alkaloids (harmaline, harmalol and harmine) in both sucrose and NaCl solutions (Table I). In contrast, similar concentrations of polyamines (1.0 mM) or cationic amino acids (10 mM) did not inhibit choline uptake by blastocysts in a statistically significant manner (data not shown). Inhibition by harmalol, harmaline and HC-3 was competitive (Figs. 2 and 3), and the K_i values for inhibition and the K_m value for choline uptake were 2–3-fold higher in 144 mM NaCl than in 288 mM sucrose (Table III).

Choline uptake by blastocysts was greater than two orders of magnitude more rapid than uptake by 1- or 2-cell conceptuses (Tables I and IV; note different units of uptake in the two tables). Thus, mediated choline uptake by 1- or 2-cell conceptuses (V_{max} values estimated to be about 0.13 fmol/conceptus per min vs. V_{max} values for blastocysts in Table III) was barely detectable by our assay in NaCl, KCl or TMAO solutions (Table IV). Nevertheless, choline uptake by 1- and 2-cell conceptuses in Mops-buffered sucrose was inhibited by the same substances that inhibited uptake by blastocysts (Tables I and IV).

Discussion

As for several amino acid transport systems [35], the activity of the system that transports choline in preim-

TABLE III

Values of kinetic parameters for choline uptake and inhibition of uptake by harmalol, harmaline and HC-3 in blastocysts

The values were determined from Hofstee plots such as those presented in Figs. 2 and 3. Where standard errors are shown, they were each calculated from three Hofstee plots produced independently from three separate sets of data. The K_m value for choline uptake was significantly higher in isotonic NaCl than in isotonic sucrose ($P < 0.01$).

Osmolite	Values of kinetic parameters ± S.E.		
	K_m [a]	V_{max} [b]	K_i [a] (inhibitor)
NaCl	61 ± 7	146 ± 22	~ 98 (harmalol) ~ 160 (harmaline) ~ 100 (HC-3)
Sucrose	22 ± 4	150 ± 11	~ 50 (harmalol) ~ 62 (harmaline)

[a] μM.
[b] fmol/blastocyst per min.

TABLE IV

Effect of various osmolites, choline analogs and harmala alkaloids on choline uptake by 1- and 2-cell conceptuses

Conceptuses were incubated with 0.67 μM [^{3}H]choline (75 Ci/mmol) and the indicated non-radioactive inhibitor for 5 min at 37°C in Mops-buffered solutions of the indicated osmolite (pH 7.2). The mean uptake ± S.E. was calculated in units of clearance of [^{3}H]choline from the medium (i.e., velocity of uptake/[choline]) utilizing five or six replicate determinations (usually five conceptuses/determination) obtained in two independent experiments. Uptake was slower in all treatment groups than in isotonic sucrose alone ($P < 0.01$). Abbreviations: HC-3, hemicholinium-3; TMAO, trimethylamine *N*-oxide.

Osmolite	Inhibitor	[^{3}H]Choline clearance (pl/conceptus per min)	
		1-cell	2-cell
Sucrose	none	24.5 ± 1.0	24.9 ± 2.5
	1.0 mM choline	1.4 ± 0.6	1.3 ± 0.4
	0.1 mM choline	2.4 ± 0.7	2.7 ± 0.5
	1.0 mM ethanolamine	0.6 ± 0.6	2.9 ± 0.9
	0.1 mM ethanolamine	5.7 ± 2.0	7.3 ± 0.6
	0.1 mM harmine	2.0 ± 0.8	2.8 ± 0.5
	0.1 mM harmaline	2.2 ± 0.8	3.8 ± 1.1
	0.1 mM harmine	1.8 ± 1.0	3.1 ± 0.9
	0.1 mM HC-3	0.7 ± 0.8	1.6 ± 0.2
TMAO	none	5.4 ± 0.9	6.0 ± 1.4
KCl	none	5.9 ± 1.1	3.0 ± 0.9
NaCl	none	6.1 ± 0.8	5.1 ± 0.7

plantation conceptuses is developmentally regulated. The latter activity increases by more than 100-fold in conceptuses between the 2-cell and blastocyst stages of development (Tables I and IV), while the external surface area increases by about 2-fold [24]. Nevertheless, this Na^+-inhibited choline transport activity can be detected as early as the 1-cell stage of development at least in sucrose-based medium (Table IV). It is conceivable that the choline transport activity increases during development of conceptuses as a result of an increase in the intracellular concentration of a monovalent cation including choline itself since choline transport is stimulated by trans exchange in red blood cells [11,20]. The maximum stimulation of choline uptake by trans exchange is, however, only about 2-fold in red blood cells at pH values around 7.2 [11]. Moreover, when conceptuses were incubated for 30 or 60 min in Brinster's medium in which the NaCl had been replaced with choline Cl (95 mM) to expand the intracellular choline pool(s), their rates of choline transport did not increase (data not shown). Choline has been shown to be incorporated into phosphatidylcholine and lysolecithin in preimplantation mouse conceptuses [36]. Moreover, omission of choline from the culture medium inhibits hatching of hamster blastocysts from their zona pellucidae in vitro [37]. Choline-containing membrane phospholipids are probably required by blastocysts both for morphological changes and for growth which begins in mouse conceptuses about 10 h prior to implantation [38]. Since choline transport increases in mouse conceptuses prior to the onset of growth, some of the choline utilized for growth might come from an exogenous source.

The characteristics of the choline transport system in conceptuses are similar to the characteristics of the Na^+-inhibited system in other mammalian cells and, except for its Na^+ inhibition, to the characteristics of the Na^+-stimulated system. Each of these systems is inhibited by ethanolamine and HC-3 (Refs. 1–12 and Tables I and IV). In addition, harmala alkaloids inhibit the Na^+-inhibited choline transport system in conceptuses, as is the case for the Na^+-stimulated system in rat striated synaptosomes [12]. It is likely, however, that these alkaloids also inhibit the Na^+-inhibited choline transport system in other cells (see Introduction). For most of the inhibitors examined in this study (other than cationic osmolites), the K_i values for competitive inhibition of choline uptake are of the same order of magnitude as the K_m value for choline transport (Refs. 1–12 and Table III). The Na^+-inhibited choline transport system in glial cells [1], placenta [6], red blood cells [20] and preimplantation conceptuses (Tables I–IV) also is inhibited by cationic osmolites including, of course, Na^+. Na^+ raises the K_m value for choline transport and the K_i values of harmaline and harmalol inhibition of choline transport by at least 2–3-fold (Table III). A completely valid statistical method has not yet appeared in the literature for estimating the S.E. of K_i values from a single graph, so the S.E. is not reported for any K_i value in Table III (an S.E. was calculated from multiple determinations of the K_m value). Nevertheless, since the K_i and K_m values determined in sucrose vs. NaCl-based media all differ by about the same magnitude (Table III), we think that it is valid to conclude that Na^+ raises the K_m and K_i values of all of these substances by the same mechanism. Since some ions need to be included in the sucrose solution in order to buffer its pH, the actual effect of cations on the K_m and K_i values may be greater than was detected in this report. These same solutions of Mops-buffered sucrose and NaCl have been used to show that, contrary to choline transport in conceptuses, choline transport in human fibroblasts is Na^+-stimulated [4].

The decrease in the K_m value for choline uptake by conceptuses when NaCl is replaced with sucrose as the major osmolite apparently cannot be attributed to an increase in the magnitude of the membrane potential (inside negative). As for red blood cells [19], the permeability of mouse oocytes and preimplantation conceptuses to Cl^- is considerably higher than their permeabilities to Na^+ or K^+ [39,40]. Thus, moving conceptuses from the NaCl-based medium in which they are stored to sucrose-based medium would lead to

depolarization rather than hyperpolarization of their plasma membranes as is the case for red blood cells (e.g., Ref. 19). For this reason, replacement of Na^+ by sucrose might underestimate the degree of inhibition of choline transport by Na^+. Since a small change in membrane potential did not have the anticipated effect on choline transport in red cells, however [20], it seems more likely that changes in membrane potential do not greatly influence choline flux.

As for the choline transporter in conceptuses, transport of cationic amino acids via systems $b^{o,+}$ and y^+ is also inhibited by Na^+ and other inorganic cations [18,19]. Similarly, the Na^+-inhibited and the Na^+-stimulated choline transport systems and amino acid transport systems $b^{o,+}$, y^+, asc and ASC are inhibited relatively strongly by the sodium site inhibitor, harmaline [17–19]. As anticipated, cationic osmolites raise the K_i value for harmaline inhibition of system $b^{o,+}$, system y^+ and the Na^+-inhibited choline transport system (Table III and Refs. 18 and 19). These characteristics of systems $b^{o,+}$, y^+, asc and ASC have been used to help to draw a parallel among the structures of their substrate receptor sites [17–19]. We suggest that this parallel should be extended to include the Na^+-inhibited and perhaps the Na^+-stimulated choline transporters at least in regard to the structures of the portions of their sites that bind choline's quaternary amino group. It is currently unclear whether the Na^+-stimulated choline transporter has one or two cation binding subsites and, if it has two such subsites, whether the two subsites have similar structures.

The choline, histidine and arginine transporters in yeast (CTR, HIP1 and CAN 1 gene products, respectively) and the cation-preferring mammalian amino acid transporter, y^+, have homologous amino acid sequences [21,22]. The cation-binding subsites in these sequences have not, however, been identified. We speculate that the amino acid sequences of the Na^+-inhibited and possibly the Na^+-stimulated choline transporters in mammalian cells may resemble the sequences of amino acid transport systems y^+, $b^{o,+}$, asc and ASC (see preceding paragraph). Since the sequence of system y^+ has recently been determined (e.g., Ref. 22) and a Na^+-stimulated choline transporter has been partially purified [41], it should soon be possible to compare the primary structures of all of these transporters both in the regions of their substrate receptor sites and in other portions of the macromolecules. In these ways, it should also become possible to compare primary structures to the functions of the transporters. It is, of course, the characteristics of the function of the transporters, such as those determined here for choline transport, that are of physiological significance to the cell. It is not yet possible, however, to predict accurately a protein's tertiary structure from its primary structure (e.g., Ref. 42) let alone confidently to predict its function from its sequence. Undoubtedly, numerous other factors, in addition to primary structure, influence tertiary structure and function especially in proteins with membrane-spanning regions. It should become easier to determine the relationships among the factors that interact to determine a protein's function when the primary structures of several related proteins with distinctly different functional characteristics have been determined. The functionally distinct but structurally related choline and amino acid transport systems discussed here appear to be a set of proteins that will become amenable to study of their structure–function relationships.

Acknowledgements

We thank Dr. Nancy Casteel for helpful comments concerning the manuscript and Barbara Le Breton for helping to prepare it. This work was supported by USPHS grant HD21801.

References

1 Hutchison, H.T., Suddith, R.L., Risk, M. and Haber, B. (1976). Neurochem. Res. 1, 201–215.
2 Wright, S.H., Wunz, T.M. and Wunz, T.P. (1992) J. Membr. Biol. 126, 51–65.
3 Tamaru, M., Iwata, M. and Nagata, Y. (1989) Neurochem. Res. 14, 607–611.
4 Riker, D.K., Roth, R.H. and Breakefield, X.O. (1981) J. Neurochem. 36, 746–752.
5 Lipton, B.A., Yorek, M.A. and Ginsberg, B.H. (1988) J. Cell. Physiol. 137, 571–576.
6 Welsch, F. (1976) Biochem. Pharmacol. 25, 1021–1030.
7 Chatterjee, T.K., Long, J.P., Cannon, J.G. and Bhatnager, R.K. (1988) Eur. J. Pharmacol. 149, 241–248.
8 Lerner, J. (1989) Comp. Biochem. Physiol. 93C, 1–9.
9 Chao, C.-K., Pomfret, E.A. and Zeisel, S.H. (1988) Biochem. J. 254, 33–38.
10 Zelinski, T. and Choy, P.C. (1984) Biochim. Biophys. Acta 794, 326–332.
11 Devés, R., Reyes, G. and Krupka, R.M. (1986) J. Membr. Biol. 93, 165–175.
12 Smart, L. (1981) Eur. J. Pharmacol. 75, 265–269.
13 Sepulveda, F.V. and Robinson, J.W.L. (1974) Biochim. Biophys. Acta 373, 527–531.
14 Dunn, M.J. and Hunt, W. (1975) J. Pharmacol. Exp. Ther. 193, 903–909.
15 Aronson, P.S. and Bounds, S.E. (1980) Am. J. Physiol. 238, F210–F217.
16 Wright, S.H. (1987) J. Membr. Biol. 95, 37–45.
17 Young, J.D., Mason, D.K. and Fincham, D.A. (1988) J. Biol. Chem. 263, 140–143.
18 Van Winkle, L.J., Campione, A.L. and Gorman, J.M. (1990) Biochim. Biophys. Acta 1025, 215–224.
19 Young, J.D., Fincham, D.A. and Harvey, C.M. (1991) Biochim. Biophys. Acta 1070, 111–118.
20 Martin, K. (1977) in Membrane transport in red cells (Ellory, J.C. and Lew, V.L., eds.), pp. 101–113, Academic Press, London.
21 Nikawa, J., Hosaka, K., Tsukagoshi, Y. and Yamashita, S. (1990) J. Biol. Chem. 265, 15996–16003.

22 Wang, H., Kavanaugh, M.P., North, R.A. and Kabat, D. (1991) Nature 352, 729–731.
23 Van Winkle, L.J., Christensen, H.N. and Campione, A.L. (1985) J. Biol. Chem. 260, 12118–12123.
24 Van Winkle, L.J., Campione, A.L., Gorman, J.M. and Weimer, B.D. (1990) Biochim. Biophys. Acta 1021, 77–84.
25 Van Winkle, L.J. and Campione, A.L. (1990) Biochim. Biophys. Acta 1028, 165–173.
26 Fowler, R.E. and Edwards, R.G. (1957) J. Endocrinol. 15, 374–384.
27 Brinster, R.L. (1971) in Pathways to Conception: The Role of the Cervix and Oviduct in Reproduction (Sherman, A.I., ed.), Charles C. Thomas, Springfield.
28 Van Winkle, L.J., Campione, A.L. and Farrington, B.H. (1990) Biochim. Biophys. Acta 1025, 225–233.
29 Van Winkle, L.J., Mann, D.F., Weimer, B.D. and Campione, A.L. (1991) Biochim. Biophys. Acta 1068, 231–236.
30 Van Winkle, L.J., Haghighat, N., Campione, A.L. and Gorman, J.M. (1988) Biochim. Biophys. Acta 941, 241–256.
31 Van Winkle, L.J., Campione, A.L. and Gorman, J.M. (1988) J. Biol. Chem. 263, 3150–3163.
32 Woolf, C.M. (1968) Principles of Biometry, 1st Edn., D. Van Nostrand, London.
33 Kramer, C.Y. (1956) Biometrics 12, 307–310.
34 Van Winkle, L.J., Mann, D.F., Wasserlauf, H.G. and Patel, M. (1992) Biochim. Biophys. Acta 1107, 299–304.
35 Van Winkle, L.J. (1992) in Mammalian Amino Acid Transport: Mechanisms and Control (Kilberg, M.S. and Häussinger, D., eds.), Plenum Press, New York, in press.
36 Pratt, H.P.M. (1980) J. Reprod. Fert. 58, 237–248.
37 Kane, M.T. and Bavister, B.D. (1988) Biol. Reprod. 39, 1137–1143.
38 Weitlauf, H.M. (1973) Anat. Rec. 176, 121–124.
39 Powers, R.D. and Tupper, J.T. (1975) Exp. Cell. Res. 91, 413–421.
40 Powers, R.D. and Tupper, J.T. (1977) Dev. Biol. 56, 306–315.
41 Knipper, M., Kahle, C. and Breer, H. (1991) Biochim. Biophys. Acta 1065, 107–113.
42 Tauber, A.I. and Sarkar, S. (1992) Persp. Biol. Med. 35, 220–235.