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Sodium-dependent carnitine transport in human placental choriocarcinoma cells

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Abstract

The JAR human placental choriocarcinoma cells were found to transport carnitine into the intracellular space by a Na^+-dependent process. The transport showed no requirement for anions. The Na^+-dependent process was saturable and the apparent Michaelis-Menten constant for carnitine was 12.3 ± 0.5 μM. Na^+ activated the transport by increasing the affinity of the transport system for carnitine. The transport system specifically interacted with L-carnitine, D-carnitine, acetyl-DL-carnitine and betaine. 6-*N*-Trimethyllysine and choline had little or no effect on carnitine transport. Of the total transport measured, transport into the intracellular space represented 90%. Plasma membrane vesicles prepared from JAR cells were found to bind carnitine in a Na^+-dependent manner. The binding was saturable with an apparent dissociation constant of 0.66 ± 0.08 μM. The binding process was specific for L-carnitine, D-carnitine, acetyl-DL-carnitine, and betaine. 6-*N*-Trimethyllysine and choline showed little or no affinity. It is concluded that the JAR cells express a Na^+-dependent high-affinity system for carnitine transport and that the Na^+-dependent high-affinity carnitine binding detected in purified JAR cell plasma membrane vesicles is possibly related to the transmembrane transport process.

Keywords: Carnitine; High-affinity transport; High-affinity binding; Plasma membrane; JAR choriocarcinoma cell; Human placenta

1. Introduction

Despite the very low ability for biosynthesis of carnitine in developing fetuses [1–4], carnitine levels in fetal circulation are significantly higher than in maternal circulation [5,6]. These observations strongly suggest that the placenta is capable of active transfer of carnitine from mother to fetus. Carnitine is an obligate requirement for fatty acid oxidation. The process of carnitine transport across the placenta is of physiological importance because this enables the fetus to build up tissue reserves of carnitine in preparation for metabolic transition which occurs at birth. While glucose is the major source of energy during prenatal development, fatty acids become the primary metabolic fuel during the immediate postnatal period. Very little is known on the cellular mechanism involved in the transfer of carnitine across the placenta from mother to fetus. Our studies with purified human placental brush-border membrane vesicles have demonstrated Na^+-dependent, high-affinity, carnitine binding in this membrane [7]. The brush-border membrane of the placental syncytiotrophoblast faces the maternal side and is in direct contact with maternal blood. We speculated that the Na^+-dependent carnitine binding identified in this membrane is involved in the active transport of carnitine from maternal blood into the syncytiotrophoblast [7].

To gain a better understanding of the placental transport of carnitine and of the relevance of the Na^+-dependent carnitine binding in the placental brush-border membrane to the transport process, we investigated carnitine transport in choriocarcinoma cells (JAR) derived from human placenta. The JAR cells have been extensively used in our laboratory as a model to study placental transport of several organic solutes including amino acids [8–11], monoamines [12], vitamins [13], and thyroid hormones [14]. The results of the current investigation reveal that these cells possess a Na^+-dependent active mechanism for carnitine transport and that the Na^+-dependent binding observed in the plasma membrane is related to the transport process.

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2. Materials and methods

2.1. Materials

L-[*methyl*-^{3}H]Carnitine (specific radioactivity, 77 Ci/mmol) was purchased from Amersham. 5-[1,2-^{3}H]Hydroxytryptamine (serotonin) (specific radioactivity, 30.4 Ci/mmol) was purchased from Du Pont-New England Nuclear. L-Carnitine, D-carnitine, acetyl-DL-carnitine, 6-*N*-trimethyllysine, β-hydroxybutyrate, γ-aminobutyrate, betaine, choline and *N*-methylglucamine were obtained from Sigma. All other chemicals were of analytical grade.

The JAR human placental choriocarcinoma cells were purchased from the American Type Culture Collection (Rockville, MD, USA). RPMI 1640 culture medium, penicillin, and streptomycin were obtained from Life Technologies. Fetal bovine serum was from Sigma.

2.2. Culture of JAR cells

The cells were cultured with RPMI 1640 medium as described previously [8]. The medium was supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Trypsin-released cells were seeded in 35-mm Petri dishes at a density of $1.5 \cdot 10^6$ cells/dish and allowed to grow as a monolayer. 24 h after subculturing, the medium was replaced with fresh culture medium, and the cells were used for uptake measurements the following day.

2.3. Transport measurements

All steps involved in transport measurements were carried out at room temperature. The medium was aspirated, and the cells were washed once with the transport buffer. 1 ml of transport buffer containing [^{3}H]carnitine was added to the cells and incubated for the desired time. Transport was terminated by aspirating the buffer and subsequently washing the cells three times with fresh uptake buffer. The cells were lysed with 1 ml of 0.2 N NaOH-1% SDS, and the lysate was transferred to scintillation vials for quantitation of radioactivity. The composition of the transport buffer in most experiments was 25 mM Hepes/Tris, pH 7.5, 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5 mM glucose. In experiments dealing with the anion dependence of carnitine transport, the composition of the transport buffer was modified by substituting potassium gluconate for KCl and calcium gluconate for $CaCl_2$ and sodium salts containing the desired anion for NaCl. In experiments dealing with the influence of Na^+ on carnitine transport, the buffers containing 140 mM NaCl or 140 mM *N*-methylglucamine chloride were mixed to give uptake buffers of desired Na^+ composition.

2.4. Differentiation between transport and binding in cells

The cells were incubated with [^{3}H]carnitine for 60 min. Total carnitine associated with the cells which represented binding plus transport was determined as described above. To determine the relative contribution of binding versus transport to the total carnitine associated with cells, the cells were lysed with 1 ml of 5 mM Tris-HCl buffer, pH 7.5, and the lysate was subjected to centrifugation at $100\,000 \times g$ for 1 h. The radioactivity associated with the pellet and with the supernatant was then determined.

2.5. Preparation of plasma membrane vesicles from cultured JAR cells

The cells were cultured in 225-cm^2 flasks and plasma membrane vesicles were prepared from the cells as described previously [15]. Briefly, the culture medium was removed by aspiration. The cells were chilled on ice and washed with 10 ml of ice-cold phosphate-buffered saline, and lysed with 25 ml of ice-cold 10 mM Tris/HCl buffer, pH 7.5, containing 2 mM EGTA. The lysate was homogenized for 30 s in an Ultra-Turrax. A stock solution of 1 M $MgCl_2$ was added to the homogenate to give a final $MgCl_2$ concentration of 10 mM. The mixture was stirred at 4°C for 10 min and centrifuged at $3000 \times g$ for 15 min. The resulting supernatant was again centrifuged at $60\,000 \times g$ for 30 min and the pellet containing plasma membranes was rinsed with and suspended in 20 mM Hepes/Tris buffer, pH 7.5, containing 150 mM mannitol and 75 mM potassium gluconate. These membrane preparations were found to be enriched in alkaline phosphatase activity (marker for plasma membrane) 8 to 10-fold compared to the homogenate.

2.6. Transport / binding measurements in membrane vesicles

Transport/binding was initiated by mixing 40 μl of membrane vesicles with 160 μl of transport/binding medium containing radiolabeled substrate (carnitine or serotonin). The composition of the transport/binding medium was 20 mM Hepes/Tris, pH 7.5 and 150 mM NaCl or KCl. The mixture was incubated at room temperature for the desired time. At the end of incubation, transport/binding was terminated by the addition of 3 ml of ice-cold 5 mM Hepes/Tris buffer, pH 7.5, containing 160 mM KCl, followed by filtration under vacuum on a Millipore Filter (DAWP type, 0.65 μm pore size). The filter, which retained the plasma membrane vesicles, was washed four times with 5 ml of the same KCl-buffer, following which the radioactivity associated with the filter was determined by liquid scintillation spectrometry.

2.7. Statistics

Experiments were routinely made in duplicate or triplicate and each experiment was repeated two to three times. The results are given as means ± S.E. Kinetic analyses were done using a commercially available computer program Fig. P. version 6.0 (BioSoft, Cambridge, United Kingdom).

3. Results

3.1. Time course of carnitine transport

Fig. 1 describes the time course of carnitine transport in JAR human placental choriocarcinoma cells in the presence of either choline chloride or NaCl in the extracellular medium. There was very little transport from the medium containing choline chloride. The presence of NaCl in the medium stimulated the transport several-fold. The NaCl-dependent transport was linear at least up to 60 min.

3.2. Dependence of carnitine transport on cations and anions

The role of different monovalent cations and anions in the transport of carnitine (5 nM or 10 μM) in JAR cells was then investigated (Table 1). Replacement of NaCl with KCl or choline chloride drastically reduced the transport, showing that Na^+ is obligatory for the transport process. There was however substantial transport of carnitine when LiCl replaced NaCl in the medium, suggesting that Li^+ can substitute for Na^+ to a significant extent. The anion influence on the transport was studied by measuring the transport in the presence of sodium salts containing different anions. Substitution of Cl^- with gluconate had no effect on the transport. Similarly, when Cl^- was replaced with NO_3^- and I^-, the effect was minimal. F^- stimulated the transport and SCN^- reduced the transport to a significant extent. The data however show that Cl^- is not obligatory for carnitine transport. Thus, the transport of carnitine in JAR cells is a Na^+-dependent process with no dependence on Cl^-.

Table 1
Influence of monovalent cations and anions on carnitine transport in JAR placental choriocarcinoma cells

Salt	Carnitine transport	
	5 nM (fmol/mg per 10 min)	10 μM (pmol/mg per 10 min)
NaCl	43.5 ± 0.5 (100)	41.5 ± 0.1 (100)
Na gluconate	49.9 ± 0.6 (115)	44.3 ± 0.1 (107)
$NaNO_3$	33.7 ± 0.3 (78)	ND
NaI	30.7 ± 0.6 (71)	ND
NaF	66.6 ± 1.6 (153)	47.7 ± 0.6 (115)
NaSCN	24.0 ± 0.1 (55)	30.0 ± 0.2 (72)
LiCl	24.7 ± 0.2 (57)	25.8 ± 0.3 (62)
KCl	5.0 ± 0.1 (12)	13.3 ± 0.2 (32)
Choline Cl	2.2 ± 0.3 (5)	5.4 ± 0.2 (13)

Transport of carnitine (5 nM or 10 μM) in monolayer cultures of JAR cells was measured with a 10 min incubation in the presence of 140 mM of indicated inorganic salts in the extracellular medium. Values are means ± S.E. Values in parentheses are percent of respective control transport measured in the presence of NaCl. ND, not determined.

3.3. Substrate specificity of the JAR cell carnitine transport system

The influence of various structurally related compounds (500 μM) on the transport of [^{3}H]carnitine (3 nM or 10 μM) was studied to determine the substrate specificity of the system that is responsible for the transport of carnitine in JAR cells (Table 2). The transport was inhibited

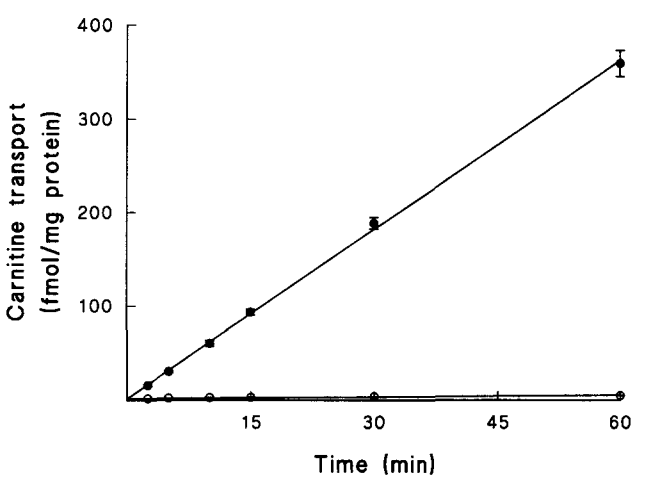

Fig. 1. Time course of carnitine transport in JAR human placental choriocarcinoma cells. Transport of carnitine (5 nM) in confluent monolayers of JAR cells was measured at pH 7.5 in the presence of either NaCl (●) or KCl (○).

Table 2
Substrate specificity of the carnitine transport system in JAR placental choriocarcinoma cells

Substrate	[^{3}H]Carnitine transport	
Analog	3 nM (fmol/mg per 30 min)	10 μM (pmol/mg per 30 min)
None	72.8 ± 2.6 (100)	129.4 ± 1.9 (100)
L-Carnitine	3.1 ± 0.1 (4)	7.3 ± 0.1 (6)
D-Carnitine	9.5 ± 0.2 (13)	40.9 ± 0.3 (32)
Acetyl-DL-carnitine	9.1 ± 0.3 (13)	30.4 ± 0.2 (24)
Betaine	29.1 ± 1.3 (40)	85.2 ± 2.1 (66)
6-*N*-Trimethyllysine	67.0 ± 1.4 (92)	ND
β-Hydroxybutyrate	71.2 ± 2.3 (98)	ND
γ-Aminobutyrate	71.2 ± 1.8 (98)	ND
Choline	71.0 ± 1.8 (98)	127.5 ± 2.2 (99)

Transport of [^{3}H]carnitine (3 nM or 10 μM) in monolayer cultures of JAR cells was measured with a 30 min incubation in the presence of Na^+. When present, the concentration of unlabeled substrate analogs was 500 μM. Values are means ± S.E. Values in parentheses are percent of respective control transport. ND, not determined.

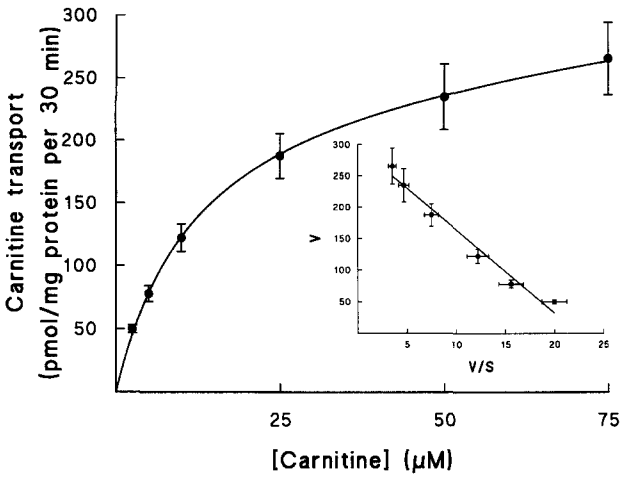

Fig. 2. Saturation kinetics of carnitine transport in JAR cells. Transport of carnitine in confluent monolayers of JAR cells was measured in the presence of NaCl with a 30 min incubation. Concentration of carnitine was varied over the range of 2.5–75 μM. Inset: Eadie-Hofstee transformation of the data for the saturable component of transport. The value for the nonsaturable component was determined by fitting the data for total transport to a transport model describing a single saturable system plus a nonsaturable component. The theoretically derived nonsaturable component was subtracted from total transport to calculate the saturable component.

markedly by L-carnitine, D-carnitine, and acetyl-DL-carnitine. As would be expected for a competitive process, the extent of inhibition by these compounds was much greater at a [^{3}H]carnitine concentration of 3 nM than at 10 μM. 6-*N*-Trimethyllysine, β-hydroxybutyrate, and γ-aminobutyrate, on the other hand, did not have any effect on carnitine transport. Betaine however caused a significant inhibition. These data show that the JAR cell carnitine transport system interacts with L- and D-carnitine and their acyl derivatives. The system also interacts with betaine, though with much lesser affinity than for carnitine.

3.4. Saturation kinetics of carnitine transport

Transport of carnitine in JAR cells was measured in JAR cells in the presence of Na^+ over a concentration range of 2.5–75 μM. The data given in Fig. 2 demonstrate that the transport system is saturable. The experimental values were found to fit best ($r = 0.9997$) for the following transport model describing a single saturable transport system plus a nonsaturable diffusion component

$$V = \frac{V_{max} \cdot S}{K_t + S} + K_D \cdot S$$

where V is the transport rate, S is the substrate concentration, K_t and V_{max} are the Michaelis-Menten constant and the maximal transport velocity, respectively, for the saturable process, and K_D is the coefficient for the nonsaturable component. This analysis gave a value of 12.3 ± 0.5 μM for K_t and 264 ± 3 pmol/mg of protein per 30 min for V_{max}. The diffusional component determined from this analysis represented 2.5% of total transport at 2.5 μM carnitine and 14% of total transport at 75 μM carnitine.

3.5. Influence of Na^+ on carnitine transport

Since the transport of carnitine in JAR cells exhibits an obligatory requirement for Na^+, the influence of Na^+ on the kinetics of carnitine transport was investigated. In these experiments, the concentration of Na^+ in the transport buffer had to be varied by isoosmotically substituting Na^+ with a suitable cation. Generally, choline or *N*-methylglucamine is used for this purpose. However, choline is structurally similar to carnitine. Even though the data in Table 2 show that choline at 0.5 mM does not interfere with carnitine transport, this may not be true at much higher concentrations of choline. Therefore, we first tested the effect of choline and *N*-methylglucamine on Na^+-dependent carnitine transport at high concentrations of these cations. It was found that choline did interfere with carnitine transport at high concentrations, the inhibition being very significant (27%) at 40 mM choline. However, *N*-methylglucamine was without effect even at this high concentration. Therefore, we used *N*-methylglucamine as the osmotic substituent for Na^+ to investigate the influence of Na^+ on carnitine transport. Fig. 3 describes the dependence of carnitine transport in JAR cells on the concentration of Na^+ in the extracellular medium. Whether the concentration of carnitine was 10 nM (Fig. 3A) or 10 μM (Fig. 3B), the transport was hyperbolically related to Na^+ concentration. The linearity of the Hill-type plot of the data (Fig. 3, insets) shows that the Na^+/carnitine coupling ratio is 1 at both concentrations of carnitine. The apparent dissociation constant for the interaction of Na^+ with the transport system was 6.8 ± 0.9 mM at 10 nM carnitine. The value was approximately the same (5.0 ± 0.3 mM) at 10 μM carnitine.

The saturation kinetics of carnitine transport were then analyzed at two different Na^+ concentrations, 5 mM and 50 mM. In these experiments, transport was measured over a carnitine concentration range of 2.5–50 μM with the [^{3}H]carnitine concentration kept constant at 20 nM. The nonsaturable component was determined by measuring the transport of radiolabel from 20 nM [^{3}H]carnitine in the presence of excess amount (1 mM) of unlabeled carnitine. The value calculated from this experimental approach agreed well with the value obtained from the theoretical approach described in Fig. 2. The nonsaturable component was subtracted from the total transport to calculate the carrier-mediated component and the data for the carrier-mediated component are given in Fig. 4A. At both concentrations of Na^+, the transport was hyperbolically related to carnitine concentration. Eadie-Hofstee transformation of the data showed that the plots were linear ($r^2 > 0.99$), indicating participation of a single saturable process under both experimental conditions (Fig. 4B). At 50 mM Na^+, the kinetic parameters K_t and V_{max} were 20.5 ± 1.0 μM

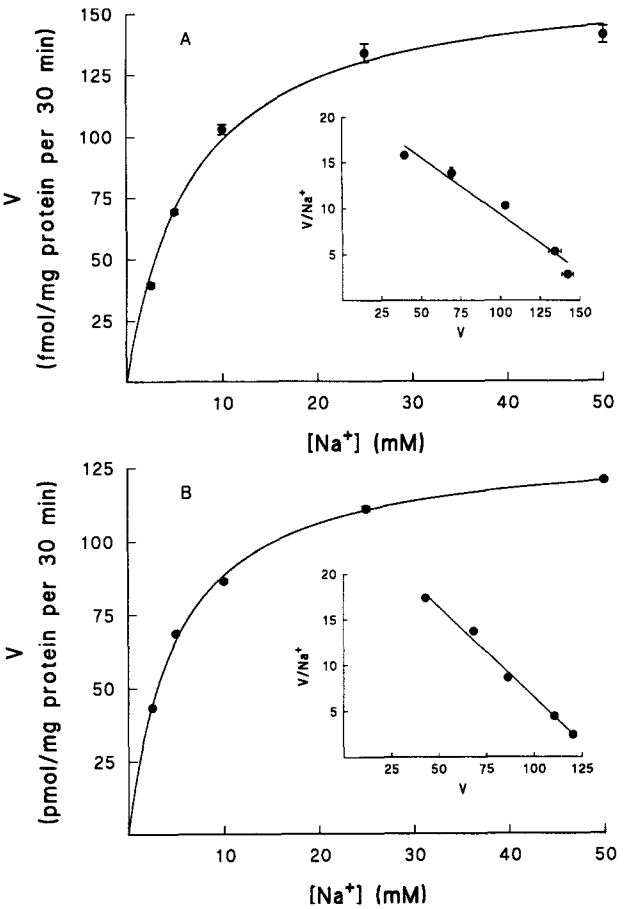

Fig. 3. Influence of Na^+ on carnitine transport in JAR cells. Transport of carnitine at two different concentrations, 10 nM (A) and 10 μM (B), was measured with a 30 min incubation in the presence of increasing concentrations of Na^+ (as NaCl) in the extracellular medium. Osmolality of the medium was adjusted by adding appropriate concentrations of *N*-methylglucamine chloride. The total concentration of NaCl plus *N*-methylglucamine chloride in the medium was 140 mM. The values for Na^+-dependent transport (i.e., transport in the presence of Na^+ minus transport in the absence of Na^+) were used to construct Hill-type plots (insets).

and 407 ± 13 pmol/mg of protein per 30 min. When the Na^+ concentration was reduced to 5 mM, the corresponding values were 36.2 ± 1.7 μM and 367 ± 13 pmol/mg of protein per 30 min. Thus, Na^+ increased the affinity of the transport process for carnitine and had no influence on the maximal transport velocity.

3.6. Transport versus binding of carnitine in JAR cells

Brush-border membrane vesicles prepared from normal placenta bind carnitine in a Na^+-dependent manner [7]. Therefore, it was of interest to determine whether or not the observed association of carnitine with intact JAR cells was due to transport into the intracellular space. The amount of carnitine associated with the cells when the cells were incubated with 10 nM carnitine for 1 h in the presence of Na^+ was 374 ± 23 fmol/dish. When the carnitine-loaded cells were lysed and the membrane component of the cells was separated from the soluble component by ultracentrifugation, 91 ± 3% of carnitine was found in the soluble fraction. These data demonstrate that carnitine was transported into the intracellular space in intact JAR cells.

3.7. Handling of carnitine in plasma membrane vesicles from JAR cells

To compare with the handling of carnitine by placental brush-border membrane vesicles, plasma membrane vesicles were prepared from JAR cells and used to study the interaction of carnitine in the absence of an intact cell system. Fig. 5A describes the association of carnitine (25 nM) with JAR cell plasma membrane vesicles in the presence or absence of an inwardly directed Na^+ gradient. With a 1 min incubation, association of carnitine with the membrane vesicles was 10.8 ± 1.1 fmol/mg of protein in the absence of a Na^+ gradient. The value increased 2.5-fold to 26.7 ± 1.5 fmol/mg of protein in the presence of a Na^+ gradient. These results show that the association of carnitine with JAR cell plasma membrane vesicles is Na^+-dependent. Interestingly, the Na^+-dependent nature of carnitine interaction with the membrane vesicles was evident even with 4 h incubation (equilibrium) when no Na^+ gradient was expected to exist across the plasma membrane. Under these conditions, the amount of carnitine associated with the vesicles was 53.7 ± 0.6 fmol/mg of protein in the absence of Na^+. This value increased 4.4-fold to 233.7 ± 4.6 fmol/mg of protein in the presence of Na^+. These findings show that the association of carnitine with the JAR cell plasma membrane vesicles was due to Na^+-dependent binding to the membrane. This binding is apparently dependent on Na^+ per se rather than a transmembrane Na^+ gradient.

To validate this conclusion, the handling of serotonin by JAR cell plasma membrane vesicles was studied under similar experimental conditions. JAR cells express a Na^+-dependent serotonin transport system [12]. Brush-border membrane vesicles from normal placenta are also able to transport serotonin into the intravesicular space in a Na^+ gradient-dependent manner [16]. Fig. 5B describes the handling of serotonin (25 nM) by JAR cell plasma membrane vesicles in the presence or absence of an inwardly directed Na^+ gradient. With a 1 min incubation, the amount of serotonin associated with the membrane vesicles was 70.6 ± 3.4 fmol/mg of protein in the absence of a Na^+ gradient. This value increased 9.1-fold to 641.4 ± 103.9 fmol/mg of protein in the presence of a Na^+ gradient. These results show that the association of serotonin with JAR cell plasma membrane vesicles is Na^+-dependent. This association was however dependent upon the transmembrane Na^+ gradient rather than Na^+ per se because, at equilibrium (4 h incubation) when there was no Na^+ gradient across the membrane, the amount of serotonin associated with the vesicles was comparable in the

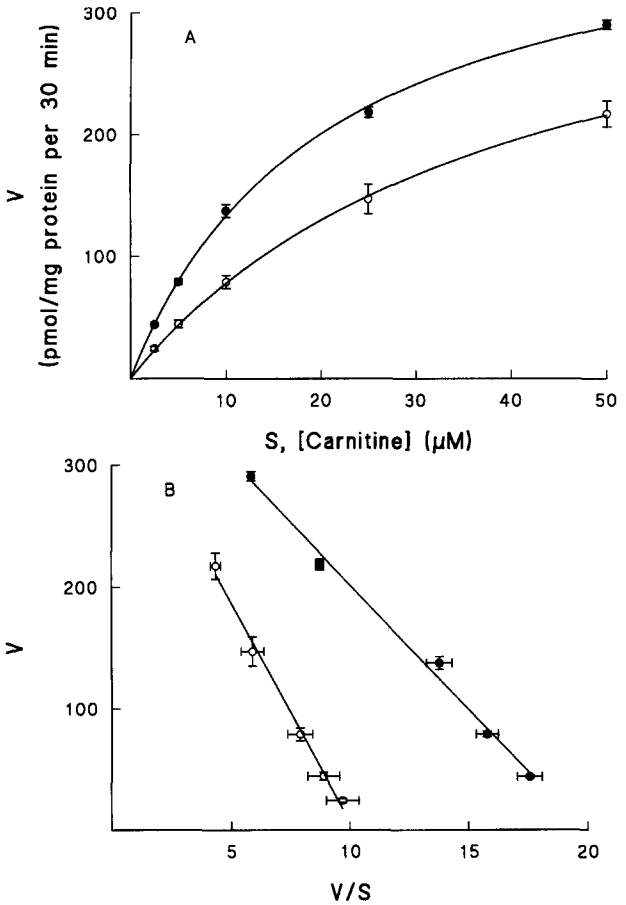

Fig. 4. Influence of Na^+ on carnitine transport kinetics in JAR cells. Transport of carnitine in JAR cells was measured with a 30 min incubation in the presence of either 50 mM Na^+ (●) or 5 mM Na^+ (○) over a carnitine concentration range of 2.5–50 μM. Osmolality of the transport buffer was maintained by appropriate addition of *N*-methylglucamine chloride. The total concentration of NaCl plus *N*-methylglucamine chloride in the buffer was 140 mM.

presence and in the absence of Na^+ (304.0 ± 6.8 versus 307.4 ± 15.9 fmol/mg of protein). Thus, there was no Na^+-dependence for serotonin association with JAR cell plasma membrane vesicles at equilibrium. These results are different from those for carnitine interaction. Furthermore, serotonin association with the membrane vesicles exhibited the 'overshoot' (i.e., the amount of serotonin associated with the vesicles in the presence of Na^+ was much higher with 1 min incubation than with 4 h incubation). These results show that the Na^+ gradient-dependent transport of serotonin observed in intact JAR cells could be demonstrated in purified JAR cell plasma membrane vesicles. In contrast, even though carnitine was transported into the intracellular space in intact JAR cells in a Na^+-dependent manner, only Na^+-dependent binding of carnitine could be demonstrated in purified JAR cell plasma membrane vesicles.

3.8. Characteristics of carnitine binding to JAR cell plasma membrane vesicles

Fig. 6 describes the saturation kinetics of carnitine binding to JAR cell plasma membrane vesicles. Equilibrium binding (4 h incubation) was measured over a carnitine concentration range of 0.25–10 μM. Nonspecific binding was determined from the binding of 25 nM [^{3}H]carnitine in the presence of excess amount (1 mM) unlabeled carnitine. This component was subtracted from total binding to calculate the specific binding. The specific binding was saturable and Scatchard analysis of the data indicated the presence of a single binding site. The apparent dissociation constant (K_d) for the interaction of carnitine with the binding site was 0.66 ± 0.08 μM and the maximal binding capacity (B_{max}) was 174 ± 9 pmol/mg of protein.

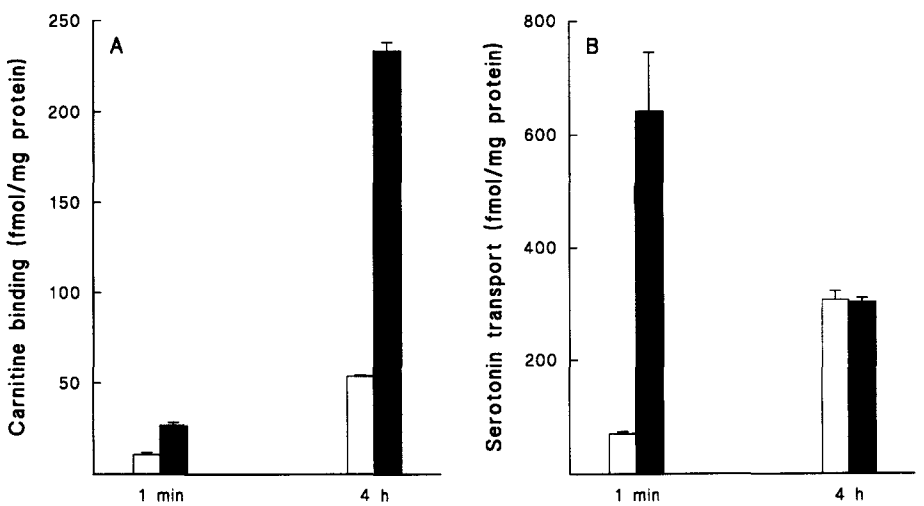

Fig. 5. Influence of Na^+ on the association of carnitine (A) and serotonin (B) with plasma membrane vesicles from JAR cells. Membrane vesicles were preloaded with 10 mM Hepes/Tris buffer (pH 7.5) containing 75 mM potassium gluconate and 150 mM mannitol. Association of carnitine (25 nM) or serotonin (25 nM) was initiated by mixing 40 μl of membrane suspension (200 μg membrane protein) with 160 μl of 10 mM Hepes/Tris buffer (pH 7.5), containing respective radiolabeled substrate and either 150 mM NaCl (closed bars) or 150 mM KCl (open bars). The mixture was incubated for either 1 min or 4 h and the amount of carnitine or serotonin that was associated with the vesicles was determined by rapid filtration assay.

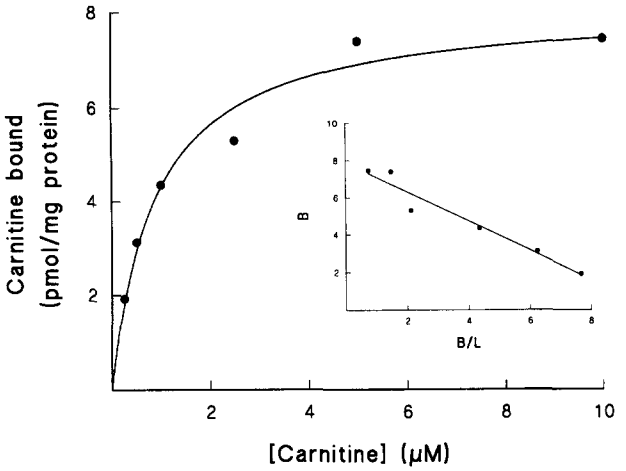

Fig. 6. Saturation kinetics of carnitine binding in JAR cell plasma membrane vesicles. Equilibrium binding of carnitine to membrane preparations was measured in the presence of Na^+ and with a 4 h incubation. Concentration of carnitine was varied over the range of 0.25–10 μM, but the concentration of radiolabeled carnitine was kept constant at 25 nM. Nonspecific binding was determined from the amount of radiolabeled carnitine bound to the membranes in the presence of excess amount (1 mM) of unlabeled carnitine. This component was subtracted from total binding to calculate the saturable specific binding. Inset: Scatchard plot of the data. B, carnitine binding in pmol/mg of protein; L, carnitine concentration in μM. Since the amount of carnitine bound to the membrane was < 1% of carnitine present during the binding assay, Scatchard analysis was done using the total carnitine concentration rather than the free carnitine concentration.

The substrate specificity of the binding process was analyzed by determining the ability of various structurally related unlabeled compounds (100 μM) to compete with 25 nM [^{3}H]carnitine for binding to the JAR cell plasma membrane vesicles (Table 3). L-Carnitine, D-carnitine, and acetyl-DL-carnitine almost totally blocked the binding of [^{3}H]carnitine. Betaine also was a very potent inhibitor. In contrast, 6-*N*-trimethyllysine was a weak inhibitor while choline was almost totally inactive.

Table 3
Substrate specificity of the carnitine binding site in JAR cell plasma membrane vesicles

Substrate analog	[^{3}H]Carnitine transport	
	(fmol/mg per 10 min)	(%)
None	118.7 ± 6.4	100
L-Carnitine	5.8 ± 0.2	5
D-Carnitine	2.7 ± 0.5	2
Acetyl-DL-carnitine	1.1 ± 0.5	1
Betaine	22.0 ± 1.0	18
6-*N*-Trimethyllysine	87.9 ± 1.9	74
Choline	111.3 ± 1.1	94

Equilibrium binding (4 h incubation) of 25 nM [^{3}H]carnitine to JAR cell plasma membrane preparations was assayed in the absence or in the presence of indicated unlabeled substrate analogs (100 μM). Nonspecific binding was determined in the presence of 1 mM unlabeled carnitine and this component was subtracted from the total binding to obtain specific binding. Values are means ± S.E.

4. Discussion

The current investigation was undertaken to characterize the transport of carnitine in intact choriocarcinoma cells of human placental origin and compare the transport process with the handling of carnitine by plasma membrane vesicles isolated from the cells. The results of the investigation show that the JAR choriocarcinoma cells are able to transport carnitine into the intracellular space in a Na^+-dependent manner. There is no involvement of anions in the process. Na^+ stimulates carnitine transport by increasing the affinity of the transport system for carnitine. The system is specific for carnitine and its acyl derivatives. Interestingly, plasma membrane vesicles isolated from these cells also exhibit Na^+-dependent binding, a process which is dependent on Na^+ rather than a transmembrane Na^+ gradient. Under identical conditions, the membrane vesicles are able to transport serotonin into the intravesicular space in response to a Na^+ gradient, ruling out experimental artifacts in the assay procedure. At equilibrium when experimentally imposed ion gradients are expected to dissipate completely, accumulation of serotonin in the vesicles shows no Na^+-dependence. In contrast, association of carnitine with the vesicles is clearly Na^+-dependent under these conditions, indicating that carnitine binds to the membranes. The binding process is saturable and is specific for carnitine and its acyl derivatives.

Comparison of carnitine transport in intact JAR cells and carnitine binding in isolated JAR cell plasma membranes reveals similarities between the two processes in important aspects. Both processes are Na^+-dependent and the substrate specificity of the processes is similar. The transport process as well as the binding process specifically interact with carnitine and its acyl derivatives but not with β-hydroxybutyrate, γ-aminobutyrate, choline, and 6-*N*-trimethyllysine. Betaine also interacts with the transport process as well as the binding process. These two processes however differ significantly in their affinity for carnitine. The apparent dissociation constant (K_t) for carnitine transport in JAR cells is 12.3 ± 0.5 μM while the corresponding value (K_d) for carnitine binding in plasma membranes is 0.7 ± 0.1 μM. There are at least three likely explanations for these findings. It is possible that the Na^+-dependent carnitine binding and the Na^+-dependent carnitine transport are mediated by two different proteins which have different affinities for carnitine. The similarities in substrate specificity between the two processes may indicate a functional relationship, but such as relationship is yet to be established. Alternatively, the binding observed in isolated plasma membrane vesicles is a functional component of the transporter itself. In intact cells, the transporter binds carnitine and mediates its transfer across the

plasma membrane. In contrast, in isolated plasma membrane vesicles the transporter binds carnitine but fails to translocate it across the membrane or the transporter mediates the transfer of carnitine following binding but fails to release it into the intravesicular space. This might happen if some cellular component is required for the completion of the transfer process. Such a component is present in intact cells but may be lost during preparation of plasma membrane vesicles. For example, transmembrane transfer of carnitine via the transporter may be functionally coupled to acylation of carnitine which requires acyl-CoA and carnitine acyltransferase. It has indeed been documented that purified placental brush-border membrane vesicles possess carnitine acyltransferase activity [17]. However, since purified plasma membrane vesicles are devoid of acyl-CoA, acylation of carnitine cannot occur which might interfere with the release of carnitine from the transporter. If this is the case, the observed difference between the K_t and the K_d values requires explanation. It is possible that the different experimental conditions employed for the determination of these two parameters contributed to the difference in these two values. The K_t value is determined from the measurements of initial transport rates in the presence of a transmembrane Na^+ gradient over a concentration range of carnitine whereas the K_d value is determined from the measurements of equilibrium binding in the presence of Na^+ but in the absence of a Na^+ gradient. The transport rate is influenced by various parameters which include the binding affinity for the substrate, the translocation of the loaded and unloaded transport protein across the membrane and functional coupling between the Na^+ gradient and the translocation process. This might explain the difference between the K_t and K_d values. The third possibility is that, even though intact cells can transport carnitine across the plasma membrane into the intracellular space, in isolated plasma membrane vesicles carnitine binds to some component other than the transporter on the internal surface of the membrane following transport. The identity of the binding component on the internal surface of the membrane however remains to be established.

The characteristics of carnitine transport in JAR cells are very similar to those found in renal brush-border membrane vesicles [18,19] and in cultured cell lines such as the neuroblastoma NB-2a cells [20]. In renal brush-border membrane vesicles, carnitine transport is Na^+-dependent with no involvement of anions. The transport process is specific for carnitine, butyrobetaine, and acetyl carnitine. One interesting difference however is that while carnitine transport/binding in the present study is inhibited by betaine, carnitine transport in renal brush-border membrane vesicles is stimulated several-fold by betaine [19]. A likely explanation for the difference may be that betaine enters the renal brush-border membrane vesicles by an independent system (e.g., the Na^+ plus Cl^- coupled betaine transporter) and subsequently causes trans-stimulation of carnitine transport via the carnitine transporter. The JAR cells may not possess this independent mechanism for betaine entry across the plasma membrane. Carnitine transport in NB-2a cells has been shown to be partially Na^+-dependent [20]. The transport process does not interact with choline or γ-aminobutyrate as has been found in the present study with JAR cells.

In conclusion, the present study describes the characteristics of carnitine transport in the human placental choriocarcinoma cell line JAR. The transport is active, Na^+ gradient-dependent, and is specific for carnitine and its close structural analogs. We speculate that this transport system may possibly be related to the carnitine binding process observed in JAR plasma membrane vesicles. The characteristics of carnitine binding to JAR cell plasma membranes are similar to those of carnitine binding to brush-border membranes prepared from normal placenta [7]. It is possible that the carnitine binding observed in placental brush-border membranes is related to the transport of carnitine from maternal blood into the syncytiotrophoblast.

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