Biochimica et Biophysica Acta, 778 (1984) 201–209
Elsevier

BBA 72324

SOME PROPERTIES OF THE THIAMINE UPTAKE SYSTEM IN ISOLATED RAT HEPATOCYTES

KUNINORI YOSHIOKA

Department of Biochemistry, Kyoto Prefectural University of Medicine, Kamikyoku, Kyoto 602 (Japan)

(Received February 8th, 1984)
(Revised manuscript received June 12th, 1984)

Key words: Thiamine transport; Quarternary ammonium compound; (Rat hepatocyte)

A kinetic study of [^{14}C]thiamine uptake over a concentration range from 0.1 μM to 4 mM was performed in isolated rat hepatocytes. The results showed that two processes contribute to the entry in rat hepatocytes: a low affinity process with a K_t of 34.1 μM and V_{max} of 20.8 pmol/10^5 cells per 30 s and a high affinity process with a K_t of 1.26 μM and V_{max} of 1.21 pmol/10^5 cells per 30 s. The uptake of thiamine by the high affinity process was concentrative and reduced in a betaine medium or K^+ medium. Both ouabain and 2,4-dinitrophenol decreased the thiamine uptake by the high affinity process. These findings indicate that the transport of thiamine via a high affinity process is dependent on Na^+ and biological energy. The uptake of thiamine was strongly inhibited by thiamine analogs such as dimethialium and chloroethylthiamine. Among quarternary ammonium compounds other than thiamine derivatives, choline and acetylcholine significantly inhibited thiamine uptake by rat liver cells, whereas betaine and carnitine did not. A kinetic study of thiamine uptake by rat hepatocytes preloaded with pyrithiamine, a potent inhibitor of thiamine pyrophosphokinase, revealed that the biphasic property of thiamine uptake disappeared and a single carrier system for thiamine with a K_t of 40.5 μM, which was similar to the K_t value of the low affinity process, was retained. These results strongly suggest that thiamine transport system in rat liver cells is closely connected with thiamine pyrophosphokinase, which accelerates the uptake rate of thiamine by pyrophosphorylation at physiological concentrations of thiamine.

Introduction

Previous papers [1–3] reported that the uptake of thiamine by isolated rat hepatocytes occurs via an active transport mechanism. Chen [1] found that thiamine is transported via an active and Na^+-dependent process, resulting in the accumulation of free thiamine in the cells, and the system was saturated by thiamine of about 1 mM; the process proceeded in a linear fashion at concentrations higher than 1 mM. On the other hand, Lumeng et al. [2] noted that thiamine accumulates rapidly in rat liver cells, but the uptake of thiamine continues to increase with time, principally owing to the accumulation of thiamine pyrophosphate, a coenzyme form of thiamine. A previous paper from our laboratory [3] demonstrated through an uptake study on dimethialium, an unphosphorylatable thiamine analog, that thiamine transport is dissociable from thiamine phosphorylation.

Although thiamine is transported via an active and Na^+-dependent process, information on the kinetics of the transport in liver cells is limited so far. Chen [1] first described the values of K_t (0.31 mM) and V_{max} (0.7 μmol/ml intracellular fluid per 5 min) of hepatic thiamine transport system using thiamine at concentrations from 10 μM to 1 mM,

much higher than the physiological concentration of thiamine, while Lumeng et al. did not perform a kinetic study on thiamine transport in isolated rat hepatocytes. Therefore, it may be necessary to investigate the kinetics of thiamine uptake by isolated rat hepatocytes and the related metabolism of thiamine at lower thiamine concentrations.

In the present paper, the evidence presented suggests that rat liver cells take up thiamine by a single carrier system, which is active and Na^+-dependent, and closely connected with thiamine pyrophosphokinase in the cytosol at physiological concentrations of thiamine. And some other properties of the specificity of the thiamine uptake system in isolated rat hepatocytes are also described.

Materials and Methods

Chemicals. [^{14}C]Thiamine ([*thiazole*-2-^{14}C]thiamine hydrochloride, 24.3 Ci/mol) was purchased from Amersham International (U.K.). The radiochemical purity of [^{14}C]thiamine was estimated to be 97.8% by paper chromatography in isopropanol/0.5 M sodium acetate buffer (pH 4.5)/water (65 : 15 : 20, v/v). No further break down of [^{14}C]thiamine was found throughout the study. The following agents were used: pyrithiamine hydrobromide, oxythiamine hydrochloride, thiamine monophosphate chloride and bovine serum albumin (Fraction V powder) (Sigma Chemical Co.); thiamine hydrochloride, thiamine pyrophosphate chloride, betaine monohydrate, carnitine chloride, choline chloride and acetylcholine chloride (Nakarai Chemical Ltd., Kyoto); and collagenase (CLS IV) from Worthington. Dimethialium (3-2′-methyl-4′-aminopyrimidyl-(5′)-methyl-4,5-dimethylthiazolium chloride hydrochloride), chloroethylthiamine (3-2′-methyl-4′-aminopyrimidyl-(5′)-methyl-4-methyl-5-chloroethylthiazolium chloride hydrochloride) and thiamine sulfuric acid ester were gifts from Takeda Chemical Industries, Ltd. (Osaka), Sankyo Co. Ltd. (Tokyo) and Tanabe Chemical Industries, Ltd. (Osaka), respectively. Thiamine acetic acid was synthesized according to the procedure of Neal [13].

Preparation of isolated hepatocytes and assay of thiamine uptake. Hepatocytes were prepared according to the procedure of Seglen [8] with minor modifications, from 250–300 g Wistar male rat fed ad libitum. Following cannulation for the portal vein, the liver was removed and perfused for 10 min with a medium containing 137 mM NaCl, 5.4 mM KCl, 0.5 mM NaH_2PO_4, 0.4 mM Na_2HPO_4, 10 mM Hepes, 0.5 mM EGTA, 4 mM $NaHCO_3$ and 5 mM glucose (pH 7.2). The perfusion was then continued for an additional 10 min with a medium containing 137 mM NaCl, 5.4 mM KCl, 0.5 mM NaH_2PO_4, 0.4 mM Na_2HPO_4, 10 mM Hepes, 4 mM $NaHCO_3$, 5 mM $CaCl_2$ and collagenase (0.5 mg/ml) (pH 7.5). The liver was minced and suspended in the Krebs-Henseleit medium containing dialyzed bovine serum albumin (25 mg/ml) (pH 7.4). The digested tissue was strained through a nylon mesh, and then the isolated cells were centrifuged at $50 \times g$ for 1 min to separate the parenchymal cells from Küpffer cells. The parenchymal cells in the pellet were resuspended and washed five times with the Krebs-Henseleit medium containing dialyzed bovine serum albumin (25 mg/ml). Cells prepared in this manner showed a viability of more than 95% by the Trypan blue exclusion method. The liver cells preloaded with pyrithiamine were prepared as followed: 10 mg/kg of pyrithiamine was injected into the rat peritoneal cavity and after 1 h, the liver was perfused with 800 ml perfusion medium containing 100 μM pyrithiamine for 20 min, and then the cell suspensions were prepared according to the procedure described above.

The transport assay of thiamine was initiated in a Corning centrifugation tube (50 ml) after preincubation for 15 min at 37°C by the addition of [^{14}C]thiamine in 3 ml of cell suspension ($3.5 \cdot 10^6$ cells/ml) in the Krebs-Henseleit medium containing dialyzed bovine serum albumin (25 mg/ml), streptomycin (100 μg/ml) and penicillin G (100 units/ml). The experiments were terminated by the addition of 15 ml ice-cold medium. After separation of the medium from the cell pellets by centrifugation for 5 s at $700 \times g$, the cell pellets were washed with 10 ml ice-cold medium, and then recentrifuged for 5 s at $700 \times g$ as described previously [3]. In the experiments on the effect of Na^+ deletion on the uptake of thiamine, the cells were centrifuged at $50 \times g$ for 15 s after preincubation for 15 min and then resuspended in the

same volume of a betaine medium or K^+ medium wherein $KHCO_3$ was substituted for $NaHCO_3$ and equiosmolar betaine monohydrate or KCl for NaCl. After the cells in the betaine medium or K^+ medium were centrifuged again at $50 \times g$ for 15 s and resuspended in the same volume of a betaine medium or K^+ medium, the transport experiments were initiated by the addition of [^{14}C]thiamine at indicated concentration. The preincubation and incubation were carried out at 37°C and the mixtures were equilibrated with 95% O_2 and 5% CO_2 at all times. Blank tubes were routinely determined as follows: [^{14}C]thiamine was added to the cell suspensions at 0°C, and then immediately diluted, centrifuged and washed by the same procedure described above. The [^{14}C]thiamine compounds were extracted by the addition of 1 ml 6.3% trichloroacetic acid to the cell pellets and their radioactivities were measured in a liquid scintillant with Triton X-100 by means of a liquid scintillation spectrometer.

Determination of intracellular water space. The intracellular water space was determined as the difference of ^3H_2O and [^{14}C]inulin distribution space in the cell pellet [3] in parallel for each experiment, and calculated to be 2.62 ± 0.245 μl/10^6 cells (mean ± S.E., $n = 40$).

Separation of thiamine compounds by paper electrophoresis. Paper electrophoresis was carried out as follows: trichloroacetic acid and lipid in 0.8 ml of the pellet extract were removed [9], and then the samples were concentrated to dryness by a rotary evaporator and dissolved in an aliquot of the solution containing $2.5 \cdot 10^{-4}$ M each of nonradioactive thiamine, thiamine monophosphate, thiamine pyrophosphate and thiamine triphosphate. Thiamine and thiamine phosphates were separated by paper electrophoresis on Whatman 540 paper in a 0.05 M sodium citrate buffer (pH 5.4) [10] at a constant current of 1.3 mA/cm for 90 min. Thiamine compounds were then detected under long wavelength ultraviolet light after spraying with an alkaline ferricyanide reagent. The bands corresponding to thiamine, thiamine monophosphate, thiamine pyrophosphate and thiamine triphosphate, respectively, were cut and immersed in 1 ml water for 1 h and the radioactivity was measured by a liquid scintillation spectrometer. The recovery of radioactivity from the samples invariably exceeded 95%.

Results

Time-course of thiamine uptake

Fig. 1 shows the time-course of the uptake of [^{14}C]thiamine by isolated rat hepatocytes. In an Na^+ medium containing an initial concentration of 10 μM, [^{14}C]thiamine increased almost linearly with time up to 1 min during incubation at 37°C and then the increase gradually slowed down. Therefore, the incubation was carried out for 30 s to measure the initial velocities of thiamine uptake. On the other hand, the uptake of [^{14}C]thiamine was insignificant at 0°C. Thiamine taken up by rat liver cells for 30 s at 37°C was present mostly as [^{14}C]thiamine (6.6 pmol/10^5 cells, 88% of the total uptake), whereas the amounts of intracellular [^{14}C]thiamine monophoshate, [^{14}C]-thiamine pyrophosphate and [^{14}C]thiamine triphosphate were 0.38 pmol/10^5 cells, 0.47 pmol/10^5 cells and 0.03 pmol/10^5 cells, respectively (5, 6 and 0.3% of the total uptake, respectively).

Thiamine uptake as a function of thiamine concentration

Fig. 2 shows the initial rate of [^{14}C]thiamine

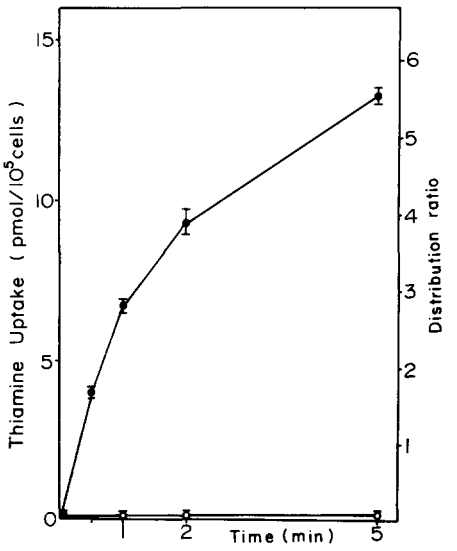

Fig. 1. Time-course of the uptake of thiamine by isolated rat hepatocytes. The uptake of 10 μM [^{14}C]thiamine was studied at 37°C (●———●) and at 0°C (○———○). The transport assays were carried out as described in the text. The results presented are the means ± S.E. of three experiments.

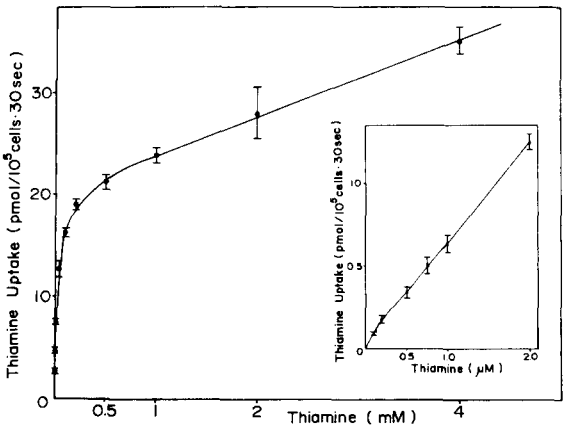

Fig. 2. Thiamine uptake as a function of thiamine concentration. Thiamine uptake was measured over a concentration range of 0.1 μM to 4 mM. Inset shows thiamine uptake as function of thiamine concentration up to 2 μM. Isolated rat hepatocytes were incubated for 30 s at 37°C as described in the text. The results presented are the means ± S.E. of three experiments.

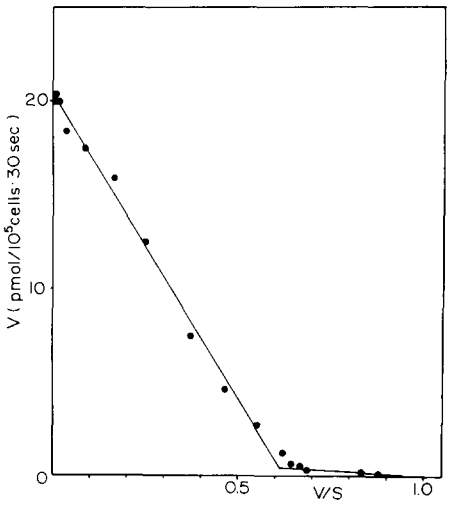

Fig. 3. Hofstee plot of thiamine uptake. Hofstee plot (V vs. V/[S]) of the data in Fig. 2 was constructed after subtraction of the contribution of the nonsaturable component from the total uptake.

uptake as a function of thiamine concentration over a concentration range from 0.1 μM to 4 mM. The curve shows that the uptake of [^{14}C]thiamine is at first saturated around 0.5 μM (inset of Fig. 2) and further the uptake of [^{14}C]thiamine increases with increasing concentrations of thiamine and reaches saturation again at 1 mM. At concentrations higher than 1 mM, the uptake of [^{14}C]thiamine was linear as reported by Chen [1]. This linear component of thiamine uptake (i.e., nonsaturable component) was assumed to be a diffusion component. Linear regression analysis was therefore performed to determine the slope of this linear portion of the plot and the amount of thiamine taken up by diffusion was calculated as the product of this slope by extracellular thiamine concentration. After correction by subtracting the contribution of the nonsaturable component from the total uptake, a Hofstee plot of the data was drawn (Fig. 3). The plot shows the involvement of two components in thiamine uptake: (1) a low affinity process with a K_t of 34.1 μM and V_{max} of 20.8 pmol/10^5 cells per 30 s, (2) a high affinity process with a K_t of 1.26 μM and V_{max} of 1.21 pmol/10^5 cells per 30 s. These results show that thiamine is taken up by the high affinity process at low concentrations (from 0.1 to 0.5 μM), and by the low affinity process at higher thiamine concentrations (above 2 μM). The characteristics of the low affinity process of thiamine transport in isolated rat hepatocytes have been reported previously in detail by Lumeng et al. [2] and our laboratory [3]. Therefore, attention will be focused on the characterization of the high affinity process.

Thiamine uptake by a high affinity process

The uptake of thiamine in an Na^+ medium containing an initial concentration of 0.4 μM [^{14}C]thiamine at 37°C is shown in Fig. 4A. The uptake increased almost linearly with time up to 1 min during incubation, and then the increase gradually slowed down. Intracellular [^{14}C]thiamine accumulated with time and a near steady-state level with an intra- to extracellular distribution ratio of 2.1 was attained at 5 min (Fig. 4B). The intracellular accumulation of [^{14}C]thiamine pyrophosphate was rapid and its level became more than twice as much as that of [^{14}C]thiamine even at 30 s of incubation. Thereafter, the accumulation of [^{14}C]thiamine pyrophosphate proceeded with time and reached a level 38-fold higher than [^{14}C]thiamine at 60 min (Fig. 4C). The intracellular accumulation of [^{14}C]thiamine monophosphate exceeded that of [^{14}C]thiamine at 15 min, but the accumulation of [^{14}C]thiamine triphosphate was very slow. The rapid intracellular accumulation of

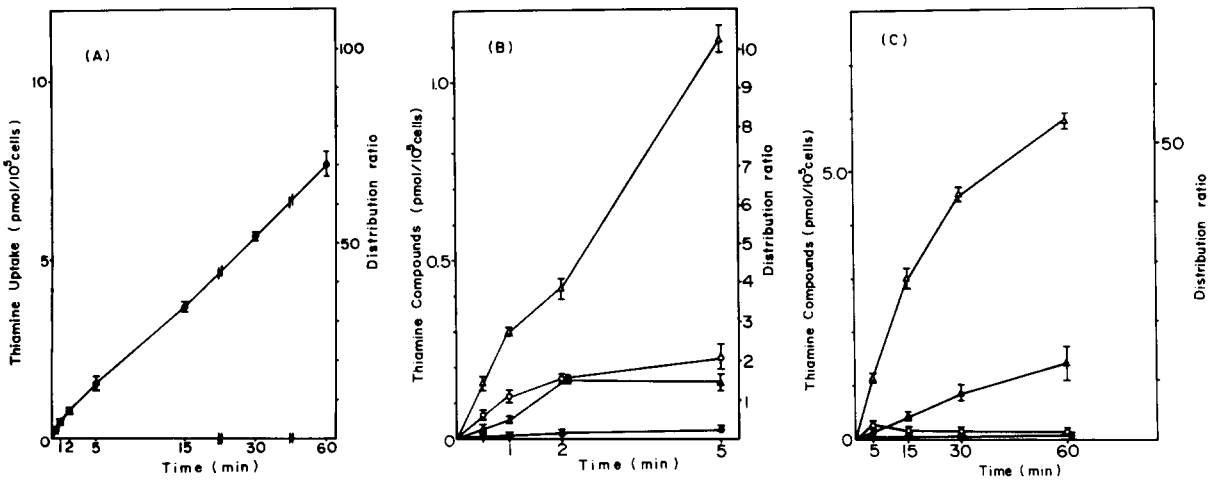

Fig. 4. The uptake and metabolism of thiamine by a high affinity process. The uptake and metabolism of thiamine in an Na^+ medium containing an initial concentration of 0.4 μM [^{14}C]thiamine in the medium was studied at 37°C. (A) The time-course of thiamine uptake for 60 min at 37°C. Cell viability after 60 min of incubation remained above 80%. (B) The intracellular amount of thiamine compounds after incubation for 5 min in an Na^+ medium. (C) The intracellular amount of thiamine compounds after incubation for 60 min. The distribution ratio is the ratio of intracellular concentration of thiamine compounds to 0.4 μM [^{14}C]thiamine in the medium. The determination of thiamine (○——○), thiamine monophosphate (▲——▲), thiamine pyrophosphate (△——△) and thiamine triphosphate (●——●) was carried out by paper electrophoresis as described in the text. The results presented are the means ± S.E. of three experiments.

thiamine phosphates (mostly thiamine pyrophosphate) found in this study is different from the results reported by Lumeng et al. who used a higher concentration of thiamine (3 μM) in the uptake medium.

In order to demonstrate that the uptake of thiamine by a high affinity process is dependent on Na^+ in the medium, [^{14}C]thiamine uptake by rat hepatocytes was studied in a betaine medium (Fig. 5B). The uptake of thiamine for 30 s in a betaine medium was 43% lower than that in an Na^+ medium. The amounts of [^{14}C]thiamine and [^{14}C]thiamine phosphates in the cells after uptake in a betaine medium were analyzed (Fig. 5A). The accumulation of [^{14}C]thiamine pyrophosphate was much higher than that of [^{14}C]thiamine after incubation for 30 s in a betaine medium. However, the intracellular level of [^{14}C]thiamine did not exceed unity even after incubation for 5 min. The intracellular [^{14}C]thiamine monophosphate level exceeded that of [^{14}C]thiamine at 1 min, whereas [^{14}C]thiamine triphosphate level was lower than that of [^{14}C]thiamine at 5 min. Also, in a K^+ medium, intracellular [^{14}C]thiamine level did not exceed unity after incubation for 5 min. These results indicate that the uptake of thiamine by a high affinity process is concentrative and dependent on Na^+ in the medium.

Effect of ouabain and 2,4-dinitrophenol on the uptake of thiamine by a high affinity process

The effects of ouabain and 2,4-dinitrophenol on thiamine uptake by a high affinity process are shown in Table I. The uptake was inhibited 37% by 0.5 mM ouabain and 56% by 1 mM 2,4-dinitrophenol. These results also indicate that the uptake of thiamine by a high affinity process is Na^+ and energy dependent.

Effect of thiamine analogs on thiamine uptake

Table II shows the effect of several thiamine analogs on [^{14}C]thiamine uptake for 30 s. Under conditions used, thiamine phosphates such as thiamine monophosphate and thiamine pyrophosphate did not inhibit [^{14}C]thiamine uptake, whereas thiamine, oxythiamine, pyrithiamine, chloroethylthiamine and dimethialium inhibited [^{14}C]thiamine uptake 52, 22, 50, 67 and 73%, respectively. Thiamine sulfuric acid ester and thiamine acetic acid were not inhibitory. Lumeng et al. [2] re-

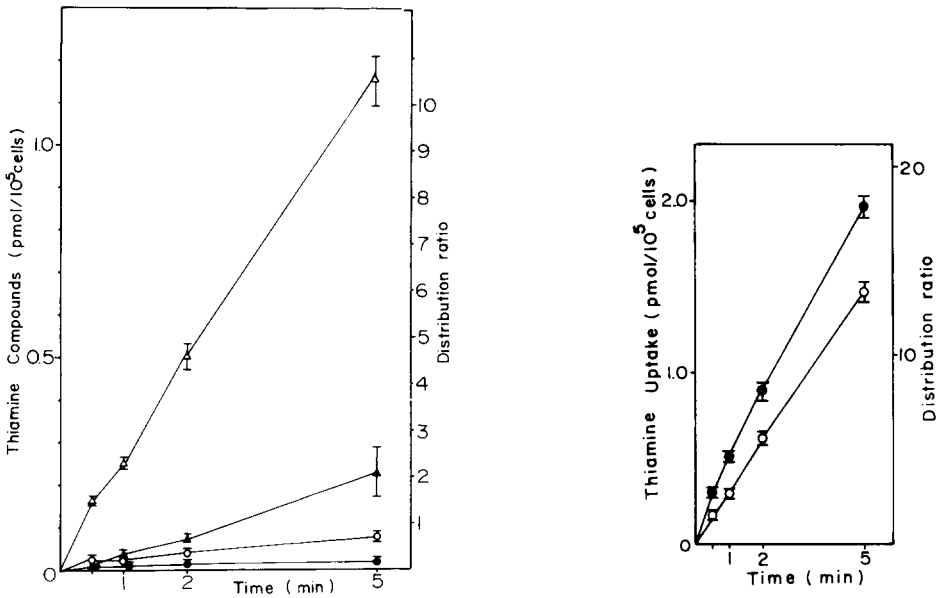

Fig. 5. The uptake and metabolism of thiamine in a betaine medium. (A) The uptake and metabolism of thiamine in a betaine medium containing an initial concentration of 0.4 μM [^{14}C]thiamine in the medium was studied for 5 min at 37°C. Cell viability remained above 80%. The distribution ratio is the ratio of intracellular concentration of thiamine compounds to 0.4 μM [^{14}C]thiamine in the medium. The determination of thiamine (○———○), thiamine monophosphate (▲———▲), thiamine pyrophosphate (△———△) and thiamine triphosphate (●———●) was carried out by paper electrophoresis as described in the text. (B) The uptake of [^{14}C]thiamine in an Na^+ medium (●———●) and in a betaine medium (○———○) for 5 min at 37°C. The results presented are the means ± S.E. of three experiments.

ported that pyrithiamine inhibited the phosphorylation of thiamine without affecting the accumulation of thiamine after incubation for 15 min. However, the addition of pyrithiamine under conditions described above affected not only the accumulation of thiamine pyrophoshate but also that of thiamine in the cells after incubation for 30 s (Table III). This indicates that pyrithiamine inhibits thiamine transport as well as thiamine phosphorylation in rat hepatocytes.

TABLE I

EFFECT OF OUABAIN AND 2,4-DINITROPHENOL ON THIAMINE UPTAKE BY A HIGH AFFINITY PROCESS

The uptake of 0.4 μM [^{14}C]thiamine was assayed as described in the text after the incubation with ouabain or 2,4-dinitrophenol for 15 min. Cell viability, based on Trypan blue exclusion, remained about 80–90%. The data presented are corrected for the contribution of nonsaturable component. The distribution ratio is the ratio of the amount of intracellular thiamine taken up to 0.4 μM thiamine in the medium. The results presented are the means ± S.E. of three experiments.

Addition	mM	[^{14}C]Thiamine uptake (pmol/10^5 cells per 30 s)	Distribution ratio	Percent (%)
None		0.209 ± 0.020	2.34 ± 0.218	100
Ouabain	0.1	0.156 ± 0.010	1.74 ± 0.114	75
	0.5	0.141 ± 0.010	1.57 ± 0.113	67
None		0.235 ± 0.008	2.59 ± 0.038	100
2,4-Dinitrophenol	0.5	0.164 ± 0.003	1.83 ± 0.034	70
	1	0.104 ± 0.009	1.16 ± 0.106	44

TABLE II

EFFECT OF THIAMINE ANALOGS ON THIAMINE UPTAKE BY A HIGH AFFINITY PROCESS

The uptake of [^{14}C]thiamine was assayed as described in the text. Nonradioactive thiamine or thiamine analog (25 μM) was added to the cell suspensions simultaneously with 0.4 μM [^{14}C]thiamine and the mixtures were incubated for 30 s. The data presented are corrected for the contribution of nonsaturable component. The distribution ratio is the ratio of the amount of intracellular thiamine taken up to 0.4 μM [^{14}C]thiamine in the medium. The results presented are the means ± S.E. of three experiments.

Addition	[^{14}C]Thiamine uptake (pmol/10^5 cells per 30 s)	Distribution ratio	Percent (%)
None	0.350 ± 0.012	2.97 ± 0.103	100
Thiamine	0.168 ± 0.011	1.42 ± 0.093	48
Thiamine monophosphate	0.344 ± 0.033	2.90 ± 0.277	98
Thiamine pyrophosphate	0.351 ± 0.013	2.98 ± 0.075	100
Pyrithiamine	0.175 ± 0.005	1.52 ± 0.005	50
Oxythiamine	0.273 ± 0.021	2.31 ± 0.177	78
Chloroethylthiamine	0.116 ± 0.014	0.98 ± 0.116	33
Dimethialium	0.096 ± 0.004	0.81 ± 0.036	27
Thiamine sulfuric acid ester	0.344 ± 0.015	2.91 ± 0.120	98
Thiamine acetic acid	0.356 ± 0.016	3.05 ± 0.130	102

Effect of quarternary ammonium compounds on thiamine uptake

In a previous paper from our laboratory [3], we showed that a choline medium was not useful in studying the dependence of Na^+ of dimethialium uptake by rat liver cells in the medium. Therefore, the effect of several quarternary ammonium compounds on thiamine uptake by rat hepatocytes was studied further. Table IV shows that betaine and carnitine which have a carboxyl group in their molecules do not inhibit thiamine uptake. On the other hand, choline and acetylcholine at concentrations of 1 mM inhibited thiamine uptake 47 and 66%, respectively. They inhibited thiamine uptake competitively and the K_i values were calculated to be 0.61 mM for choline and 0.31 mM for acetylcholine, respectively (data not shown). These results suggest that choline and acetylcholine, which have no negatively charged group in their molecules, can bind competitively to the thiamine carrier of isolated rat hepatocytes.

Effect of preloading of rat hepatocytes with pyrithiamine on thiamine uptake

From the finding described above, it was thought that a reason for the biphasic phenomenon shown in Fig. 3 might be the phosphorylation of thiamine by thiamine pyrophosphokinase,

TABLE III

EFFECT OF PYRITHIAMINE ON THIAMINE TRANSPORT AND METABOLISM IN ISOLATED RAT HEPATOCYTES

The accumulation of intracellular [^{14}C]thiamine and the further phosphorylation to thiamine pyrophosphate for 30 s of incubation in the presence or absence of pyrithiamine were measured by paper electrophoresis as described in the text. The data were obtained after subtracting the contribution of nonsaturable component from the total uptake. The distribution ratio is the ratio of the intracellular concentration of thiamine compounds to 3 μM thiamine in the medium. The results are the means ± S.E. of three experiments.

Addition	μM	[^{14}C]Thiamine		[^{14}C]Thiamine pyrophosphate	
		pmol/10^5 cells per 30 s	distribution ratio	pmol/10^5 cells per 30 s	distribution ratio
None		1.19 ± 0.110	1.79 ± 0.165	0.231 ± 0.010	0.347 ± 0.015
Pyrithiamine	15	0.973 ± 0.064	1.46 ± 0.097	0.042 ± 0.012	0.063 ± 0.018
	30	0.766 ± 0.134	1.15 ± 0.201	0.020 ± 0.002	0.030 ± 0.003
	60	0.674 ± 0.035	1.01 ± 0.053	0.028 ± 0.008	0.042 ± 0.012

TABLE IV

EFFECT OF QUARTERNARY AMMONIUM COMPOUNDS ON THIAMINE UPTAKE BY A HIGH AFFINITY PROCESS

The uptake of [^{14}C]thiamine was assayed as described in the text. Quarternary ammonium compounds (1 mM) were added to the liver cell suspensions simultaneously with 0.4 μM [^{14}C]thiamine and the mixtures were incubated for 30 s. The data are corrected for the contribution of nonsaturable component. The distribution ratio is the ratio of the amount of intracellular thiamine taken up to 0.4 μM [^{14}C]thiamine in the medium. The results presented are the means $\pm$ S.E. of three experiments.

Addition	[^{14}C]Thiamine uptake (pmol/10^5 cells per 30 s)	Distribution ratio	Percent (%)
None	0.203 $\pm$ 0.006	2.48 $\pm$ 0.072	100
Betaine	0.208 $\pm$ 0.009	2.55 $\pm$ 0.110	102
Carnitine	0.211 $\pm$ 0.005	2.57 $\pm$ 0.065	104
Choline	0.108 $\pm$ 0.002	1.31 $\pm$ 0.028	53
Acetylcholine	0.069 $\pm$ 0.003	0.86 $\pm$ 0.016	34

closely involved in a low affinity process; this results in an increase in the rate of thiamine uptake at low thiamine concentrations (0.1–0.5 μM). Therefore, a kinetic study of [^{14}C]thiamine uptake for 30 s of incubation over a concentration range from 0.1 to 20 μM was performed using cell suspensions prepared from rat liver preloaded with pyrithiamine, a potent inhibitor of thiamine pyrophosphokinase. After subtracting the diffusion component from the total uptake, the biphasic pattern shown in Fig. 3 disappeared and a single carrier system for thiamine with a K_t of 40.5 μM and V_{max} of 28.8 pmol/10^5 cells per 30 s was retained (data not shown). The K_t value obtained above closely agreed with the K_t value of the low affinity process of thiamine uptake in Fig. 3.

Discussion

Chen [1] first reported that the process of hepatic transport of thiamine was active and Na^+-dependent, with an apparent K_t of 0.31 mM and V_{max} of 0.7 μmol/ml intracellular fluid per 5 min. The values of K_t and V_{max} were determined over a concentration range from 10 μM to 1 mM which was much higher than physiological concentrations of thiamine. Lumeng et al. [2] also reported on the active transport of thiamine at a concentration of 3 μM in the medium, which was still one order of magnitude higher than that of blood plasma, but they did not perform a kinetic study on thiamine uptake.

In this paper, the kinetics of thiamine uptake by rat hepatocytes were studied over a concentration range from 0.1 μM to 4 mM, and the results show that there are two processes for thiamine uptake by rat liver cells, one with a high K_t value (34.1 μM) and the other with a low K_t value (1.26 μM). Although the low affinity process of hepatic thiamine uptake was previously characterized in detail [2,3], the K_t value of the low affinity process obtained in the present study was approximately one-tenth of the value reported by Chen [1], and it was consistent with the value of K_t for dimethialium uptake described in our previous paper [3]. A kinetic study using cell suspensions prepared from rat liver preloaded with pyrithiamine shows that the biphasic pattern disappeared and there is a single carrier system for thiamine uptake with a K_t value (40.5 μM) similar to that of the low affinity process (34.1 μM) of an active and Na^+-dependent uptake of thiamine. The reason for the biphasic phenomenon seen in Fig. 3 might be that thiamine pyrophosphokinase is closely connected with the thiamine uptake system (a low affinity system in Fig. 3). Thus, thiamine taken up in concentrations up to 0.5 μM is rapidly phosphorylated by thiamine pyrophosphokinase with a K_m of 0.45 μM for thiamine [5] in the cytosol, resulting in an increase in the initial rate of thiamine uptake. In other words, thiamine pyrophosphokinase might be involved in the thiamine uptake system to trap thiamine transported in the form of thiamine pyrophosphate and make the uptake of thiamine more effective at physiological concentrations of thiamine (0.1–0.2 μM) [11,12].

The rapidity of the accumulation of thiamine pyrophosphate in liver cells shown in Fig. 4 is

different from that of Lumeng et al. [2]. This might be due to the difference of the concentration of thiamine used in the experiments. Furthermore, Lumeng et al. studied the effect of pyrithiamine on thiamine transport and metabolism after 15 min of incubation. Under the conditions used in their experiments, thiamine transport reached a steady-state level and it was therefore difficult to know whether pyrithiamine inhibited thiamine transport or not. During the uptake of thiamine for 30 s, pyrithiamine inhibited not only the phosphorylation of thiamine but also thiamine transport, as shown in Table III.

Both thiamine monophosphate and thiamine pyrophosphate at a concentration of 25 μM inhibited thiamine uptake only slightly in a medium containing 0.4 μM thiamine, although thiamine, dimethialium and chloroethylthiamine strongly inhibited thiamine uptake. This supports our previous results [7] in which thiamine phosphates were found to be practically unavailable for liver cells. It was also suggested that thiamine phosphates have a low affinity for a carrier in the thiamine uptake system because of a negative charge of phosphate group at C-5 in the thiazole moiety. Similarly, thiamine sulfuric acid ester and thiamine acetic acid, which have a negatively charged group at C-5 in the thiazole moiety, were not inhibitory on thiamine uptake by rat liver cells.

Herzberg and Lerner [4] reported that choline inhibited thiamine transport in the small intestine. In the present study, choline and acetylcholine were strong inhibitors of thiamine uptake by rat liver cells. On the other hand, betaine and carnitine which are quarternary ammonium compounds having a carboxyl group in their molecules did not inhibit thiamine uptake. These results suggest that not only quarternary nitrogen but also net charge in the molecule is involved in the binding of these compounds to a carrier in the thiamine uptake system of hepatocytes. Choline and acetylcholine inhibited thiamine uptake competitively, and the K_i value of the thiamine uptake system for choline was much higher than the K_t value for choline uptake by liver previously reported [6]. Further kinetic study will be needed to clarify mutual interaction of these two uptake system.

Acknowledgements

The author is grateful to Professor A. Iwashima for many helpful discussions, suggestions and the critical review of the manuscript. The author also wishes to thank Dr. H. Nishimura and Dr. R. Yamada for helpful discussion and encouragement.

References

1 Chen, C.-P. (1978) J. Nutr. Sci. Vitaminol. 24, 351–362
2 Lumeng, L., Edmondson, J.W., Schenker, S. and Li, T.K. (1979) J. Biol. Chem. 254, 7265–7268
3 Yoshioka, K., Nishimura, H. and Iwashima, A. (1983) Biochim. Biophys. Acta 732, 308–311
4 Herzberg, G.R. and Lerner, J. (1973) Biochim. Biophys. Acta 307, 234–242
5 Mano, Y. (1960) J. Biochem. 47, 24–36
6 Steven, H.Z., David, L.S., Richard, J.W. and Henri, B. (1980) Proc. Natl. Acad. Sci. USA 77, 4417–4419
7 Yoshioka, K., Nishimura, H., Sempuku, K. and Iwashima, A. (1983) Experientia 39, 505–507
8 Seglen, P.O. (1972) Exp. Cell Res. 74, 450–454
9 Itokawa, Y. and Cooper, J.R. (1970) Methods Enzymol. 18A, 91–92
10 Patrini, C. and Rindi, G. (1980) Int. J. Vitam. Nutr. Res. 50, 10–18
11 Burch, H.B., Bessey, O.A., Love, R.H. and Lowry, O.H. (1952) J. Biol. Chem. 198, 477–490
12 Rindi, G., De Giuseppe, L. and Sciorelli, G. (1968) J. Nutr. 94, 447–454
13 Neal, R.A. (1969) J. Biol. Chem. 244, 5201–5205