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ACTIVE TRANSPORT OF DIMETHIALIUM IN ISOLATED RAT HEPATOCYTES

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The uptake of dimethialium, a thiamine analog having a methyl group in place of the hydroxyethyl group in the thiazole moiety, was studied in freshly isolated rat hepatocytes. In an Na^+-medium, dimethialium at 10 μM was accumulated rapidly by the cells and an almost steady intra- to extracellular distribution ratio of 4.2 was attained in 5 min of incubation. The K_t and the V_{max} for the saturable component were estimated to be 27 μM and 19 pmol/10^5 cells per min, respectively. In a K^+ medium, the uptake of dimethialium was decreased to 58% of that of control. Ouabain and 2,4-dinitrophenol significantly lowered the rate of dimethialium uptake. Both phenylthiazinothiamine and oxythiamine were inhibitory on the uptake of dimethialium, which uptake was also inhibited by choline. These data indicate that dimethialium transport in liver cells proceeds via a carrier-mediated active process dependent on Na^+ and biological energy. Furthermore, these results also suggest that thiamine transport in liver is dissociable from thiamine phosphorylation.

Although evidence has been accumulated which shows the uptake of thiamine by the isolated rat hepatocytes [1,2], small intestine [3,4], central nervous system [5,6], red blood cells [7], Ehrlich ascities tumor cells [8,9] and microorganisms [10,11] to occur by a specific, carrier-mediated transport process, the exact nature of energy coupling in the thiamine transport system is still unknown and the possible role of phosphorylation in thiamine transport has been argued [12,13]. Recently, two reports have appeared on thiamine transport in isolated rat liver cells, in which thiamine is transported by an active, Na^+-dependent process, but some discrepancies exist between these papers. Chen [1] reported that thiamine transported remained largely unmetabolized even after prolonged incubation, while Lumeng et al. [2] showed that the uptake of thiamine continued to increase with time, principally owing to the accumulation of thiamine pyrophosphate. Thus, the relationship between thiamine transport, accumulation and phosphorylation in liver cells as well as in other animal tissues is still indefinite, although Lumeng et al. [2] reported that thiamine transport and phosphorylation could be differentiated by the appropriate thiamine antagonists.

Using dimethialium, an unphosphorylatable thiamine analog [14], we have already shown that thiamine can be transported without obligatory phosphorylation in yeast cells [15], since the analog was found to accumulate by the same transport mechanism as for thiamine. Therefore, it seemed to be of interest to investigate the transport of dimethialium in isolated rat hepatocytes for the further characterization of thiamine transport in liver.

Hepatocytes were prepared according to the procedure of Seglen [16] with minor modification, from 200–300-g Wistar male rats fed ad libitum. Cells prepared in this manner showed the viability more than 95% by the Trypan blue exclusion method. The transport experiments were initiated after 15 min of preincubation at 37°C by the addition of dimethialium (obtained from Takeda

Chemical Industries, Ltd., Osaka) or [^{14}C]thiamine (24.3 mCi/mmol, Amersham International, U.K.) in 3 ml of cell suspension ((3.9–4.8)· 10^6 cells/ml) in 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 of ice-cold medium containing dialyzed bovine serum albumin. After separation of the medium from the cell pellets by the centrifugation for 5 s at 700 × g, the pellets were washed with 10 ml of ice-cold medium containing dialyzed bovine serum albumin, and then recentrifuged for 5 s at 700 × g. This washing step effectively removed most of dimethialium or [^{14}C]thiamine from the extracellular space in the cell pellets. 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. Dimethialium in the cell pellets was measured fluorimetrically as thiochrome, as previously reported [15], and the radioactivity of [^{14}C]thiamine was extracted by the addition of 1.0 ml 6.7% trichloroacetic acid to the cell pellets and measured in liquid scintillant with Triton X-100 by means of liquid scintillation spectrometer. The intracellular water space was determined as the difference of ^3H_2O and [^{14}C]inulin distribution space in the cell pellets [17] in parallel for each experiment, and was calculated to be 2.73 ± 0.16 μl/10^6 cells (mean ± S.E., n = 17).

Fig. 1 shows the time-course of the uptake of dimethialium by isolated rat hepatocytes. In an Na^+ medium, dimethialium was rapidly taken up by isolated hepatocytes and the distribution ratio of intra- to extracellular dimethialium concentration was 4.2 at 5 min of incubation at 37°C, whereas it was insignificant at 0°C. The results indicate that the dimethialium uptake by isolated rat hepatocytes is dependent on temperature and is concentrative. The 1-min rate of dimethialium uptake was measured over a concentration range from 5 μM to 2 mM. Under these conditions, the rates increased in a curvilinear manner for dimethialium, indicating the presence of both saturable and nonsaturable components as shown by the uptake of other nutrients into hepatocytes (data not shown). The K_t and the V_{max} for the saturable component was estimated by using a linear regression analysis to be 27 μM and 19

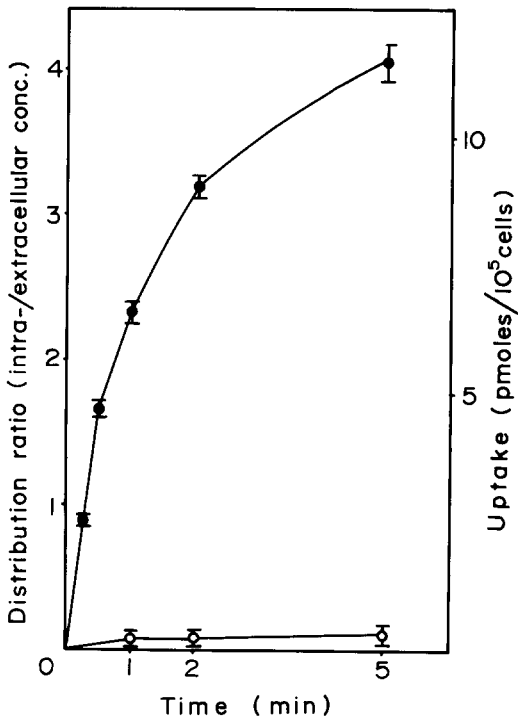

Fig. 1. Time-course of the uptake of dimethialium by isolated rat hepatocytes. The uptake of 10 μM dimethialium was studied at 37°C (●) and 0°C (○). The results presented are the means ± S.E. of the three experiments.

pmol/10^5 cells per min, respectively.

Table I shows the inhibition of the uptake of dimethialium by ouabain and 2,4-dinitrophenol. The uptake inhibited 36% by 1 mM ouabain and 42% by 1 mM 2,4-dinitrophenol. The dependence of dimethialium uptake on Na^+ in the medium was demonstrated by a decreased dimethialium uptake when Na^+ was replaced by K^+. The medium containing choline was inhibitory, but it was found that dimethialium uptake was inhibited by choline itself as previously reported on thiamine transport in small intestine [18]. Therefore, we could not use choline medium to study the dependence of dimethialium uptake on Na^+ in the medium, which differed from the results of Lumeng et al. [2].

The dimethialium uptake at the concentration of 10 μM was inhibited 41% and 28% by 0.1 mM phenylthiazinothiamine [19] and oxythiamine, respectively, which are thiamine antagonists known not to be converted to thiochrome in the fluorimetric determination of dimethialium. Furthermore,

TABLE I

EFFECT OF INHIBITORS ON DIMETHIALIUM UPTAKE BY ISOLATED RAT HEPATOCYTES

Cell viability, based on Trypan blue exclusion, remained about 80–90%. The transport assays were carried out for 1 min after preincubation for 15 min at 37°C. The results presented are the means ± S.E. of three experiments. 2,4-DNP, 2,4-dinitrophenol.

Addition	(mM)	Dimethialium uptake (pmol/10^5 cells per min)	Distribution ratio	Percentage
None		7.03 ± 0.55	2.44 ± 0.16	100
Ouabain	0.5	5.09 ± 0.14	1.77 ± 0.04	72
	1	4.47 ± 0.26	1.53 ± 0.08	64
None		6.38 ± 0.26	2.34 ± 0.08	100
2,4-DNP	0.5	4.58 ± 0.23	1.68 ± 0.78	72
	1	3.70 ± 0.16	1.36 ± 0.05	58
Na^+ medium		6.08 ± 0.22	2.39 ± 0.08	100
K^+ medium		3.52 ± 0.31	1.38 ± 0.31	58

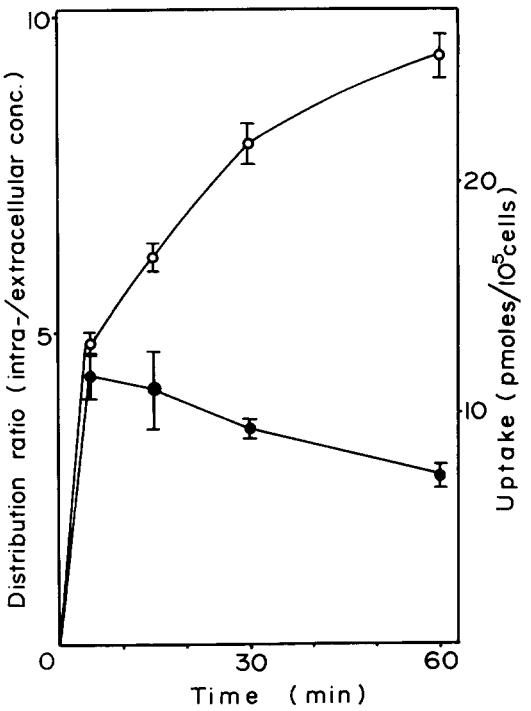

Fig. 2. Time-course of the uptake of dimethialium and [^{14}C]thiamine. The uptake of 10 μM dimethialium (●) and [^{14}C]thiamine (○) were studied at 37°C. Cell viability after 60 min of incubation remained above 80%. The results presented are the means ± S.E. of the three experiments.

[^{14}C]thiamine transport in hepatocytes was competitively inhibited by dimethialium and K_i for the saturable component was calculated to be 12 μM, which was near to the value of K_t for dimethialium uptake. These results suggested that dimethialium is taken up by liver cells through a transport system specific for dimethialium or thiamine.

However, there was a marked difference between the time-course of dimethialium uptake and that of [^{14}C]thiamine uptake (Fig. 2). At 5 min of incubation, the ratio of dimethialium uptake was nearly equal to that of [^{14}C]thiamine uptake. From 5 min to 15 min of incubation, dimethialium uptake remained at an almost steady level; thereafter a gradual loss of accumulated dimethialium, that is, an overshoot phenomenon, was observed.

On the other hand, [^{14}C]thiamine uptake continued to increase with time and reached distribution ratios more than 3-times those of dimethialium after 60 min of incubation. The continued accumulation of hepatic thiamine appeared to be due to phosphorylation of thiamine, which cannot occur with dimethialium. This accumulation of phosphate esters of thiamine in hepatocytes after prolonged incubation for 60 min was established by the method of paper electrophoresis as described by Patrini and Rind [20], indicating that the concentrations of thiamine, TMP, TPP and TTP were 6.8, 3.3, 10.3 and 0.6 pmol/10^5 cells, respectively. These results agree with those of Lumeng et al. [2], who found that thiamine accumulated mainly as thiamine pyrophosphate after 60 min of incubation.

From these findings, we conclude that dimethialium, which has the thiamine structure but is incapable of phosphorylation by thiamine pyrophosphokinase, is transported in freshly isolated rat liver cells by an Na^+-dependent active process, independently of phosphorylation. These results also strongly suggest that thiamine can be transported without obligatory phosphorylation in liver and further accumulation is achieved by its intracellular pyrophosphorylation to thiamine pyrophosphate.

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