FULL PAPER *Parasitology*

Glucose Uptake Activity in Murine Red Blood Cells Infected with *Babesia microti* and *Babesia rodhaini*

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ABSTRACT. The glucose uptake activity in *Babesia rodhaini* and *B. microti* - infected red blood cell (IRBC) was investigated in mice using 2-deoxy-D-glucose (2DOG) and L-glucose (L-Glc), a non-metabolizable analogue of D-glucose and non-incorporative glucose to non-infected RBC (NRBC), respectively. The uptake activities of both DOG and L-Glc were higher in IRBCs than those in NRBC. The concentration dependent uptake of 2DOG and L-Glc in both IRBC revealed a linear curve, indicating non-transporter mediated uptake. In addition, *B. microti* IRBC showed higher 2DOG uptake than *B. rodhaini* IRBC, whereas no difference was observed in L-Glc uptake. These results indicated that some new glucose uptake system, at least two systems, developed in both IRBC. The new systems were sodium independent, non-competitive to L-Glc, and sensitive to temperature. One of two systems had no kinetical difference between *B. rodhaini* and *B. microti* IRBC, however another one might have higher uptake activity in *B. microti* IRBC compared to that in *B. rodhaini* IRBC.

KEY WORDS: *Babesia microti*, *Babesia rodhaini*, glucose uptake, murine, RBC.

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Babesia rodhaini and *B. microti*, the major causative protozoa of babesiosis in mice, are classified into the same genus and appear morphologically quite similar each other. However, various differences have been observed between them, particularly on their glucose metabolism. *Babesia microti* shows high activity of tricarboxylic acid (TCA) cycle enzymes, suggesting to utilize mainly aerobic pathway, whereas *B. rodhaini* to utilize anaerobic pathway for glucose metabolism [16, 17]. Our previous report also demonstrated that mitochondorial function in *B. microti* was higher than that in *B. rodhaini* [18].

It has been well known that intracellular protozoa *Babesia* species depend on their substrates of glucose metabolism for the host cell [10, 13]. Since mature red blood cell has limited capabilities of these substances synthesis, almost all of them are transported from extracellular fluid. The glucose uptake into infected red blood cell (IRBC) is the most important factor for the glucose metabolism in *Babesia* protozoa.

In this regard, glucose uptake activity was investigated in *B. rodhaini* and *B. microti* IRBC using 2-deoxy-D-glucose (2DOG) and L-glucose (L-Glc), those of which are non-metabolizable analogue of D-glucose and non-incorporative glucose to non-infected red blood cell (NRBC), respectively.

MATERIALS AND METHODS

Reagents: Reagents and their suppliers were [^{3}H]-2DOG (44.0 mCi/mM) from Amersham (Amersham, UK), [^{3}H]L-Glc (14.6 mCi/mM) and [^{3}H]L-glutamic acid (41.1 mCi/mM) from New England Nuclear DUPONT (Boston, MA, U.S.A.), and N-[2-Hydroxyethyl] piperaine-N'-[2-ethanosulfonic acid] (HEPES), [2-Hydroxyethl] trimethyl-ammonium chloride(choline chloride), Sigmacell Type 50, α-cellulose (cellulose powder), L-Glc, t-octylphenoxypolyethoxyethanol (triton X-100), and 3-[4-hydroxyphenyl]-1-[2,4,6-trihydroxyphenyl]-1-propanone (phloretin) from Sigma Chemicals Co. (St. Louis, MO, U.S.A.). All other reagents were of purified grade from Wako Chemicals (Osaka, Japan).

Preparation of Babesia IRBCs: Male ICR (6–8 wk old) mice were purchased from SLC Inc. (Shizuoka, Japan). Mice were injected once intraperitoneally with *B. rodhaini* or *B. microti* infected whole blood (approximate 1×10^4 IRBC/head). Both IRBC were collected from the mice at the high parasitemia stage (approximate 50–60%) by cardiac puncture, and NRBC were collected as a control. Red blood cells (RBC) were diluted in HEPES balanced solution (HBS:145 mM NaCl, 10 mM KCl, 1 mM $MgSO_4$, 10 mM HEPES, pH 7.2), and washed (1,200 g, at 4°C, for 5 min) twice and passed through a cellulose column to remove white blood cells [12]. Eluted RBC was washed twice with HBS and diluted to adjust the percent cell volume at 25%. The cell number of adjusted IRBC and NRBC were counted with an hemocytometer.

Glucose uptake: The uptake of glucose in IRBC and NRBC were measured at 37°C using substrates, 2DOG and L-Glc. Briefly, 10 μl of the cell suspension was pre-warmed at 37°C for 10 min and added the same volume of pre-warmed HBS containing 2DOG or L-Glc (final concentration at 5 mM) plus radio labeled compounds (final specific

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activity at 5 μCi/m*l*). The cells were incubated at 37°C for varying time. After the termination of the incubation, 200 μ*l* of stop solution containing 200 μM phloretin, which is a specific inhibitor for glucose transporter 1 (GLUT1), was added and washed twice (1,450 g, for 15 sec) with the same solution. After aspiration of the supernatant, 100 μ*l* of 1% (v/v) triton X-100 and the same volume of 5% (w/v) trichloroacetic acid (TCA) were added to the cell pellet. Samples were left overnight at 4°C and spun (2,350 g, for 4 min) with a microcentrifuge. The supernatant fractions (150 μ*l*) were taken into Ready Cap (Beckman Instruments, Inc., Fullerton, CA, U.S.A.), and dried at 45–55°C. After the drying, radioactivity was counted with a scintillation counter.

For the experiment for concentration dependent 2DOG and L-Glc uptake, IRBC and NRBC were diluted in HBS containing the various concentrations of substrates (0.1, 0.5, 1.0, 2.5, 5.0, and 10 mM for 2DOG, and 0.082, 0.41, 0.82, 2.0, 4.1 and 8.2 mM for L-Glc) and incubated at 37°C for 10 min. Some of characteristics of the glucose uptake were also examined. Briefly, the uptake of glutamic acid, the similar molecular weight to L-Glc, was measured for evaluation of molecular size on the glucose uptake in IRBC. Five millimolar of L-Glc and labeled compound in HBS were replaced the same volume of glutamic acid and the half specific activity of [^{3}H]L-glutamic acid, respectively. For the measurement of inhibitory effect of L-Glc on 2DOG uptake in IRBC and NRBC, L-Glc (–) HBS and L-Glc (+) HBS (containing 100 μM L-Glc) were used. The osmotic pressures of L-Glc (–) HBS was adjusted to 310 mosM with sucrose. For sodium dependent glucose uptake, Na (–) HBS (145 mM choline chloride, 10 mM KCl, 1 mM $MgSO_4$, 10 mM HEPES-Tris, pH 7.2), in which 145 mM NaCl was replaced 145 mM choline chloride, were used. The effect of incubation temperature at 4°C on the glucose uptake was also examined. The cell suspensions were pre-incubated at 37°C followed by left on ice and incubated in pre-cooled substrate solutions on ice.

RESULTS

Glucose uptake: The time-course of 2DOG and L-Glc uptake in IRBCs and NRBC are shown in Fig. 1. The uptake rates of 2DOG and L-Glc in both IRBC were significantly higher than those in NRBC. The rate of 2DOG uptake in *B. microti* IRBC was moderately higher than that in *B. rodhaini* IRBC (Fig. 1 A), whereas no difference of L-Glc uptake was observed between them (Fig. 1B). The concentration dependent uptake of 2DOG and L-Glc in *B. rodhaini* and *B. microti* IRBC, and NRBC are shown in Fig. 2. The uptake in IRBCs and NRBC showed a saturation curve with increasing of 2DOG concentrations, however the specific uptake of 2DOG, which subtracted NRBC uptake from IRBC's, showed a linear curve. The specific uptake of 2DOG in *B. microti* IRBC tended to be higher than that in *B. rodhaini* IRBC (Fig. 2A). On the concentration dependent L-Glc uptake in both IRBC and NRBC, linear increasing curves were observed in IRBCs and also in NRBC. The uptake rate in both IRBC was extremely higher than that in NRBC. No difference of specific uptake of L-Glc was observed between *B. microti* and *B. rodhaini* IRBC (Fig. 2 B).

The time-course of L-glutamic acid uptake and the effect of L-Glc on 2DOG uptake in IRBCs and NRBC are shown in Fig. 3. No uptake of L-glutamic acid was observed in both IRBC and NRBC (Fig. 3A) and no difference of 2DOG uptake was observed among them on the condition of the absence and presence of L-Glc (Fig. 3B). Sodium dependent uptake and the effect of the incubation condition at 4°C on 2DOG and L-Glc uptake in IRBCs and NRBC are shown in Fig. 4. No change of both 2DOG and L-Glc uptake was observed in both IRBCs and NRBC on the codition of the presence and absence of sodium ion (Fig. 4A) and no uptake of 2DOG and L-Glc in IRBCs and NRBC were detected on the condition at 4°C (Fig. 4B).

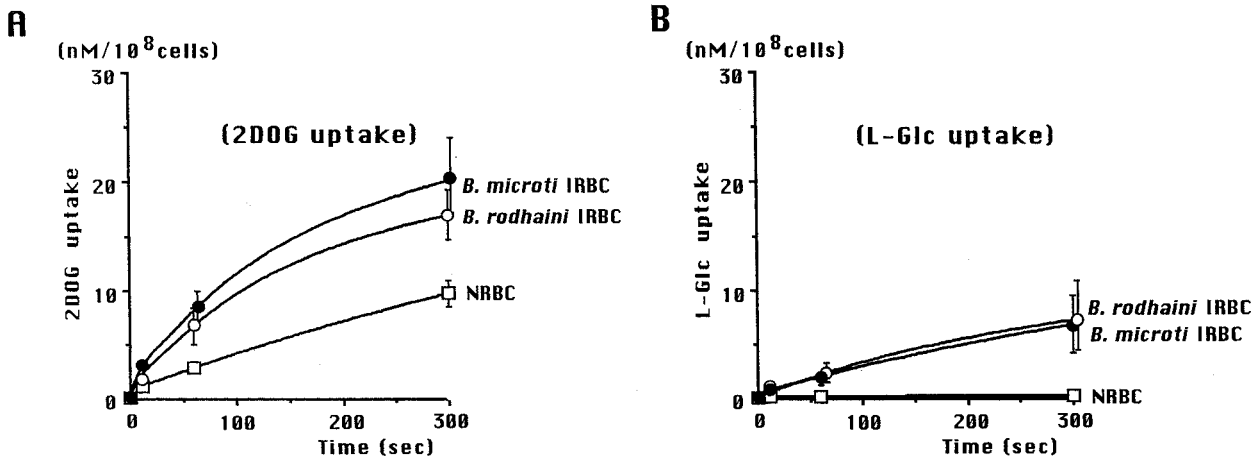

Fig. 1. The time-course of 2DOG (A) and L-Glc (B) uptake in *B. rodhaini* and *B. microti* IRBC and NRBC on the condition at 37°C. The values at each point are the mean ± SD for 3 experiments.

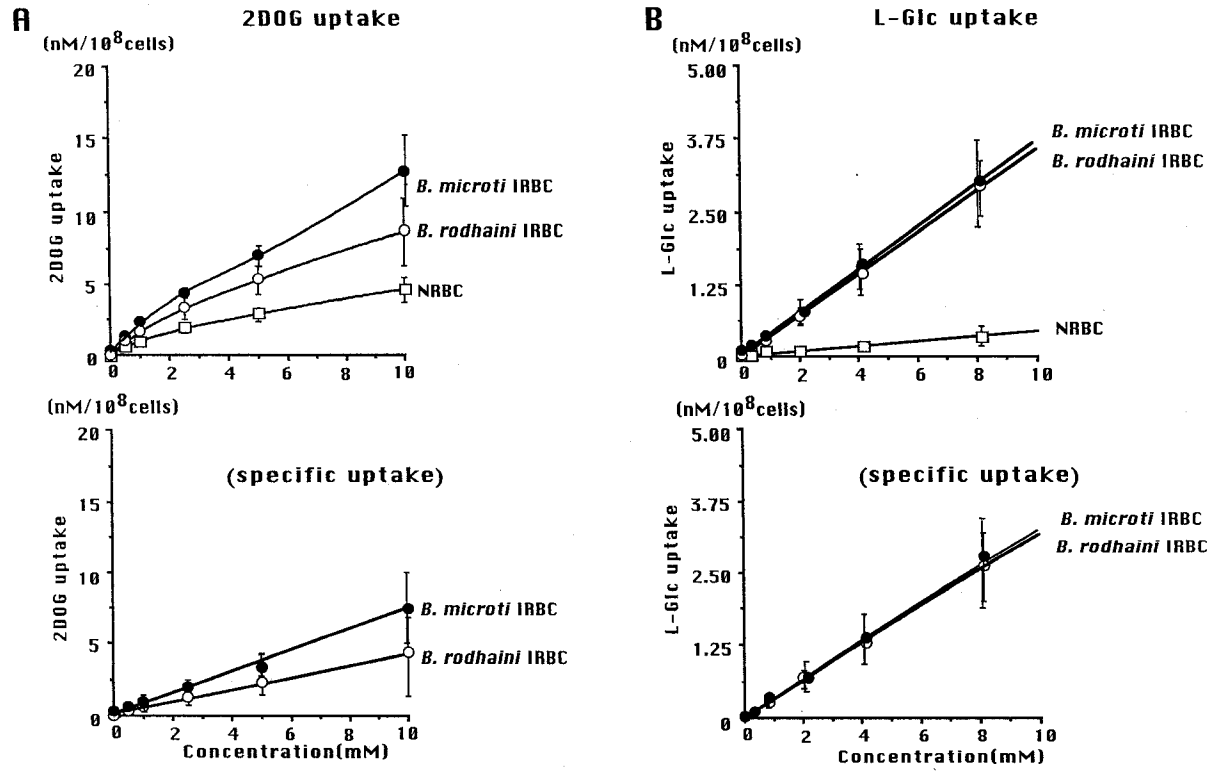

Fig. 2. Concentration dependent 2DOG (A) and L-Glc (B) uptake in *B. rodhaini* and *B. microti* IRBC, and NRBC on the condition at 37°C for 10 min. The specific uptake rates (lower panel) are subtracted NRBC from IRBC uptake. The values at each point are the mean ± SD for 3 experiments.

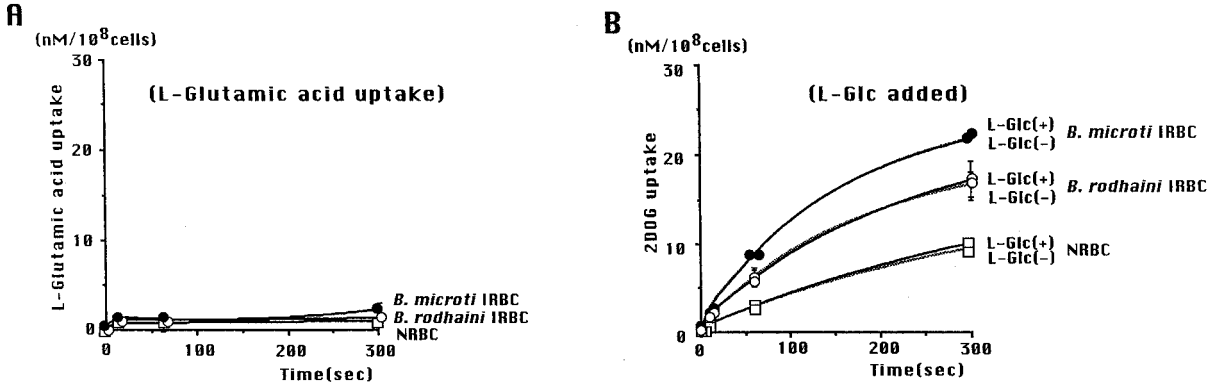

Fig. 3. The time-course of L-glutamic acid uptake (A) and the effect of L-Glc (100 μM) on 2DOG uptake (B) in *B. rodhaini* and *B. microti* IRBC and NRBC on the condition at 37°C. The values at each point are the mean ± SD for 3 experiments.

DISCUSSION

The specific uptake rates of 2DOG and L-Glc in both *B. microti* and *B. rodhaini* IRBC were significantly higher than those in NRBC. In previous reports on malaria, *Plasmodium*-infected RBC showed remarkable increase of the glucose uptake [14, 15] and also L-Glc uptake, which cannot be incorporated in NRBC [6]. Intraerythrocytic protozoa, such as *Babesia* and *Plasmodium*, were considered to enhance the glucose uptake in RBC. Our previous reports demonstrated various differences in glucose metabolism were observed between *B. rodhaini* and *B. microti*, particularly, *B. microti* utilized aerobic pathway, whereas *B. rodhaini* used anaerobic pathway [16, 17]. The relative contribution of mitochondoria to the overall energy production is larger in *B. microti* compared with that in *B. rodhaini* [18]. These differences in glucose metabolism between the two *Babesia* protozoa might relate to the uptake rate of 2DOG in IRBC.

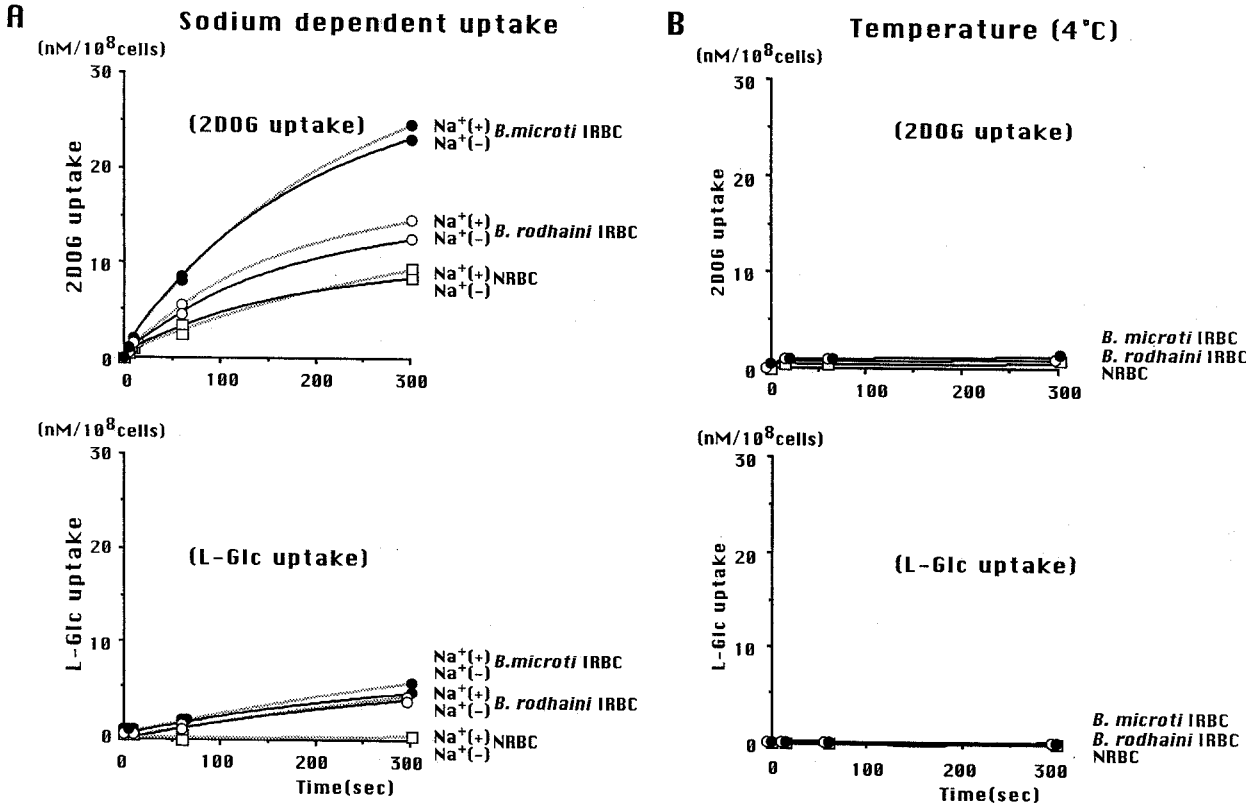

Fig. 4. Sodium dependent 2DOG and L-Glc uptake (A) and the effect of temperature (4°C) on 2DOG and L-Glc uptake (B) in *B. rodhaini* and *B. microti* IRBC and NRBC on the condition of at 37°C for sodium dependent and at 4°C. The values at each point are the mean for 2 experiments for sodium dependent and 3 for at 4°C.

Since L-Glc, an isomer of D-glucose, was incorporated in both IRBC, infected protozoa induced a new glucose uptake system in RBC. In addition, no difference of its rate was observed between *B. rodhaini* and *B. microti* IRBC, unlike the 2DOG uptake rate. This result suggested that the other system increased 2DOG uptake developed in IRBC.

Desai and co-wokers [2] suggested approximate 11Å diameter of 'pore', by which some molecules similar size to glucose could pass through, was existed in IRBC plasma membrane. Moreover, in *Plasmodium* spp. infection, various transport systems including 'metabolic window' [1], 'ducts' [2, 3, 5, 9, 11], and the combination of simple diffusion and transporter model [7, 8, 19] has been suggested in IRBC from the findings on the increase of glucose permeability with kinetical and morphological changes [4, 6]. On the concentration dependent uptake of 2DOG, NRBC showed a saturation curve, suggesting a glucose transporter, however both IRBC revealed a linear curve in specific uptake, suggesting non-transporter mediated system. The rate of concentration dependent 2DOG uptake in *B. microti* IRBC tended to be higher than that in *B. rodhaini* IRBC, whereas no difference of the L-Glc uptake rate was observed between them. Therefore, two new glucose uptake systems induced in both IRBC by Babesia infection, one of which was similar between them, and another might be different on the activity.

Many kind of channels and transporters were enhanced their activities by sodium ion. No difference was observed in the rate of 2DOG and L-Glc uptake in IRBC on the condition of the presence and absence of sodium ion. The both new 2DOG and L-Glc uptake system induced by protozoa infection were considered sodium independent systems. No difference was observed in the rate of 2DOG uptake in both IRBC on the condition of the absence and presence of L-Glc. The L-glutamic acid, similar molecular weight to L-Glc (L-glutamic acid: 147.13, L-Glc: 180.2), was not incorporated into both IRBC. Therefore, the new uptake systems were not competitive system and also have not 'pore' structure, because the system could recognize D-glucose from L-Glc. In the condition of incubation temperature at 4°C, active transport and endocytosis were remarkably suppressed, only exception of a simple diffusion. Both 2DOG and L-Glc uptake in IRBC was completely suppressed on the incubating condition at 4°C. The new glucose uptake systems induced by *Babesia* were sensitive to temperature.

From these results, it is suggested that at least two new glucose uptake systems, characterized by sodium independent, non-competitive to L-Glc, sensitive to temperature,

and not 'pore' structure, develop in IRBCs, one of which might have high transport activity in *B. microti* IRBC compared with that in *B. rodhaini* IRBC.

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