

myo-Inositol is an osmolyte in rat liver macrophages (Kupffer cells) but not in RAW 264.7 mouse macrophages

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The role of *myo*-inositol as an osmolyte was studied in cultured rat liver macrophages (Kupffer cells). Hyperosmotic exposure of Kupffer cells stimulated *myo*-inositol uptake and led to an increase in the mRNA levels for the sodium/*myo*-inositol co-transporter (SMIT). Conversely, hypo-osmotic (205 m-osM) exposure diminished *myo*-inositol uptake when compared with normo-osmotic (305 m-osM) control incubations. The hyperosmolarity-induced SMIT mRNA increase was counteracted by added *myo*-inositol or betaine. In contrast with Kupffer cells, there was only a slight hyperosmotic stimulation of *myo*-inositol uptake in RAW 264.7 mouse macrophages, and the *myo*-inositol transporter (SMIT) mRNA was not detectable. Further, a slight stimulation of taurine uptake and an increase in taurine transporter (TAUT) mRNA level by hyperosmolarity

was observed in RAW 264.7 cells, whereas hypo-osmolarity led to a decrease in taurine uptake and TAUT mRNA level. When Kupffer cells were preloaded with *myo*-inositol, hypo-osmotic exposure led to a rapid efflux of *myo*-inositol from the cells. *Myo*-inositol efflux was also stimulated by phagocytosis of latex particles; however, latex was without effect on the hyperosmolarity-induced increase of SMIT mRNA levels. The results suggest a role of *myo*-inositol as an osmolyte in rat Kupffer cells but not in RAW 264.7 mouse macrophages. The functional relevance of this osmolyte strategy might lie in the maintenance of cell volume homeostasis during phagocytosis in Kupffer cells; however, the interplay with the other osmolytes betaine and taurine remains to be established.

INTRODUCTION

Organic osmolytes are organic compounds that are specifically accumulated by or released from cells in response to aniso-osmotic cell shrinkage or cell swelling respectively, in order to maintain their volume. Osmolytes need to be non-perturbing solutes that do not interfere with protein function even when occurring at high intracellular concentrations [1–7]. Such a prerequisite might explain why only a few classes of organic compounds, namely polyols (such as *myo*-inositol and sorbitol) methylamines (such as betaine and α -glycerophosphorylcholine) and certain amino acids such as taurine have evolved as osmolytes in living cells. In mammals, increased uptake of the osmolyte *myo*-inositol in response to hyperosmolarity has been described in renal medulla cells, lens epithelia, astrocytes and endothelial cells [8–11]. The hyperosmolarity-induced *myo*-inositol accumulation inside these cells is the result of an increase in the V_{\max} of the sodium/*myo*-inositol co-transporter (SMIT) [12] and an increased expression of its gene [13]. Disturbance in accumulation of *myo*-inositol in brain is an abnormality that is found in Down syndrome and hepatic encephalopathy [14,15]. Recent studies have identified betaine as an important osmolyte in Kupffer cells [16] and RAW 264.7 macrophages [17] and taurine in Kupffer cells [18] and H4IIE hepatoma cells [19]. The respective osmolyte transporters BGT1 and TAUT are induced in response to hyperosmolarity, whereas hypo-osmotic exposure leads to a rapid efflux of betaine and taurine from the cells, probably via volume-activated anion channels [16–19]. As in many cells (reviewed in [20]), Kupffer cell function is critically controlled by cell volume [16,21–23]; the availability of betaine and taurine as osmolytes was shown to interfere strongly with important

Kupffer cell functions such as phagocytosis [18,22] or the production of eicosanoids [16,18] and tumor necrosis factor α [23]. The present study shows that in addition to betaine and taurine, *myo*-inositol is also used as an osmolyte by Kupffer cells but interestingly not by RAW 264.7 macrophages.

MATERIALS AND METHODS

Materials

Polybead fluorescent microspheres (2.5% solids latex, 1 μ m in diameter) were obtained from Polysciences Limited (St. Goar, Germany). RPMI 1640 medium (without Phenol Red) and fetal bovine serum (FBS) for culture of Kupffer cells were from Biochrom (Berlin, Germany), Dulbecco's modified Eagle's medium and FBS for culture of RAW 264.7 cells were from Gibco (Eggenstein, Germany). Guanidine thiocyanate and SDS were from Fluka (Karlsruhe, Germany). Oligonucleotide-labelling kit was from Pharmacia (Freiburg, Germany). [α -³²P]dCTP (3000 Ci/mmol) was from ICN (Meckenheim, Germany), and *myo*-[³H]inositol (22.3 Ci/mmol), [³H]taurine (24 Ci/mmol) and [¹⁴C]betaine (48.1 mCi/mmol) were from New England Nuclear-DuPont (Bad Homburg, Germany). Hybond-N nylon membranes were from Amersham Buchler (Braunschweig, Germany). The salts required for preparation of the Krebs-Henseleit buffer were from Merck (Darmstadt, Germany) and all other chemicals were from Sigma (Deisenhofen, Germany). Two plasmids containing SMIT [13] and betaine γ -aminobutyric acid transporter (BGT-1) [24] cDNAs were kindly provided by Dr. H. Moo Kwon (Division of Nephrology, The John Hopkins Uni-

Abbreviations used: BGT-1, betaine γ -aminobutyric acid transporter; DIDS, 4,4'-di-isothiocyanatostilbene-2,2'-disulphonic acid; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LDH, lactate dehydrogenase; SMIT, sodium/*myo*-inositol co-transporter; TAUT, taurine transporter.

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versity School of Medicine, Baltimore, MD, U.S.A.) To identify Kupffer cells and RAW 264.7 cells expressing mRNA for TAUT, a cDNA probe was isolated as described [19]. The 1.0 kb cDNA fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was used for standardization, was from Clontech (Palo Alto, CA, U.S.A.).

Isolation and culture of Kupffer cells

Rat Kupffer cells were prepared by collagenase–pronase perfusion and separated by a single Nycodenz gradient and centrifugal elutriation as described previously [21,25]. Cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated FBS for 48 h. The experiments were performed during the following 24 h with Krebs–Henseleit hydrogen carbonate buffer (pH 7.4) containing 10 mM glucose. The osmolarity of the medium was varied by changing the NaCl concentration. The viability of Kupffer cells was more than 95% as assessed by Trypan Blue exclusion. Kupffer cell volume was measured by flow resistance cytometry with a Casy-1 cell counter and analyser system (Schärfe Systeme, Reutlingen, Germany). In normo-osmotic medium, the average Kupffer cell volume was 724 ± 24 fl ($n = 7$). Protein content was 0.03 ± 0.01 mg/ 10^6 cells ($n = 7$). Assuming a water content of 80% of whole Kupffer cell volume, a mean intracellular water space of $23.9 \mu\text{l}/\text{mg}$ protein is estimated. Viability of the incubations was routinely tested by lactate dehydrogenase (LDH) release at the end of the incubations and was less than 15 units/l.

Determination of phagocytic activity

The phagocytic activity of Kupffer cells, i.e. the ability to ingest fluorescent latex particles, was assessed by measuring the cell-associated fluorescence [26]. Kupffer cells were incubated with RPMI 1640 medium for 48 h. Thereafter the medium was replaced by hypo-osmotic (205 m-osM), normo-osmotic (305 m-osM) or hyperosmotic (405 m-osM) Krebs–Henseleit buffer for 20 h at 37 °C in cluster 24 dishes (Costar, Cambridge, MA, U.S.A.). Osmolarity was altered by varying the NaCl concentration in the medium. Phagocytosis of Kupffer cells was measured after exposure of the cells to 0.025% fluorescein-coupled latex particles (1 μm in diameter) for 5, 30, 60 and 120 min in air/CO₂ (19:1) at 37 °C in the same medium. In additional experiments the influence of *myo*-inositol on phagocytic activity was examined. Here, 10 or 100 μM *myo*-inositol was added 4 h before the addition of latex particles. After the incubation the cells were washed four times with Krebs–Henseleit buffer and were removed from each well by using a rubber ‘policeman’. The fluorescence of cell suspension was measured with a Perkin–Elmer LS5B fluorimeter with excitation at 436 nm and emission above 500 nm. The cell-associated fluorescence was expressed as arbitrary units. Control plates were used for microscopy. The percentage of cells phagocytosing latex was greater than 95%.

Culture of RAW 264.7 cells

RAW 264.7 cells (ATCC TIB 71) were grown to near-confluency under air/CO₂ (19:1) in Dulbecco’s modified Eagle’s medium, 37 °C, pH 7.4, supplemented with 10% (v/v) FBS in Cluster 6 dishes (Costar). Unless indicated otherwise, the osmolarity was varied by changing the NaCl concentration in the medium. For identification of TAUT, BGT-1 and SMIT mRNA levels, cells were maintained under various osmotic test conditions for 12 h.

Northern blot analysis

Total RNA from near-confluent plates of Kupffer cells or RAW 264.7 cells was isolated by using guanidine thiocyanate solution as described [27]. RNA samples were subjected to electrophoresis in 0.8% (w/v) agarose/3% (v/v) formaldehyde and then blotted on Hybond-N nylon membranes with $20 \times \text{SSC}$ (3 M NaCl/0.3 M sodium citrate). After a brief rinse with water and cross-linking (Hoefer UV-crosslinker 500; Hoefer, San Francisco, CA, U.S.A.), the membranes were observed under UV to determine RNA integrity and the location of the 28 S and 18 S rRNA bands. Blots were then subjected to a 3 h pre-hybridization at 43 °C in 50% deionized formamide, in sodium phosphate buffer (0.25 M, pH 7.2), containing 0.25 M NaCl, 1 mM EDTA, 100 $\mu\text{g}/\text{ml}$ salmon sperm DNA and 7% (w/v) SDS. Hybridization was performed in the same solution with approx. 10^6 c.p.m./ml [α -³²P]dCTP-labelled random-primed SMIT, TAUT, BGT1 or GAPDH cDNA probes. Membranes were washed three times in $2 \times \text{SSC}/0.1\%$ SDS for 15 min, twice in sodium phosphate buffer (25 mM, pH 7.2)/EDTA (1 mM)/0.1% SDS for 10 min and twice in sodium phosphate buffer (25 mM, pH 7.2)/EDTA (1 mM)/1% SDS for 10 min at 53 °C. Blots were then exposed to Kodak AR X-omat film at –70 °C with intensifying screens. Suitably exposed autoradiograms were then analysed with densitometry scanning (PDI, New York, NY, U.S.A.) to determine the optical densities of the mRNA levels. Relative mRNA levels were determined by standardization of the optical density of GAPDH mRNA.

Uptake and efflux of osmolytes

For measuring the uptake of osmolytes, Kupffer cells and RAW 264.7 cells were incubated in 0.5 ml of hypo-osmotic (205 m-osM), normo-osmotic (305 m-osM) or hyperosmotic (405 m-osM) Krebs–Henseleit buffer with 10 μM or 100 μM and 0.5 $\mu\text{Ci}/\text{ml}$ *myo*-[³H]inositol, [³H]taurine or [¹⁴C]betaine for the durations indicated at 37 °C in cluster 24 dishes (Costar, Cambridge, MA, U.S.A.). Osmolarity was altered by varying the NaCl concentration in the medium. The cells were then rinsed four times with 1 ml of ice-cold stop solution [10 mM Tris/Hepes (pH 7.4)/300 mM mannitol/300 mM NaCl] and dried in air at room temperature. The cells were then harvested with 1 ml of 1% (w/v) SDS and aliquots were taken for liquid-scintillation counting and for protein determination (Bio-Rad protein assay; Bio-Rad, Hercules, CA, U.S.A.).

For studies on *myo*-inositol efflux, Kupffer cells were pre-incubated for 12 h in hyperosmotic bicarbonate-buffered Krebs–Henseleit saline (405 m-osM, being prepared by addition of 50 mM NaCl) in cluster 24 dishes (Costar), to induce *myo*-inositol transport activity. The cells were then incubated in hyperosmotic Krebs–Henseleit buffer (405 m-osM) containing 10 μM and 0.5 $\mu\text{Ci}/\text{ml}$ *myo*-[³H]inositol to load the cells with the radioisotope. After a loading period of 4 h at 37 °C under air/CO₂ (19:1), the cells were rinsed three times with hyperosmotic (405 m-osM) Krebs–Henseleit buffer. Osmolarity was altered by varying the NaCl concentration in the medium. The cells were then incubated for 0–120 min in *myo*-inositol-free hyperosmotic (405 m-osM) or hypo-osmotic (205 m-osM) Krebs–Henseleit buffer. In studies on phagocytosis, Kupffer cells were also incubated for 0–20 min in *myo*-inositol-free hyperosmotic (405 m-osM) buffer containing latex (0.025%, 1 μm in diameter). For inhibitor studies, 0.1 mM 4,4’-diisothiocyanatostilbene-2,2’-disulphonic acid (DIDS) was added during the last 15 min of the loading period and the entire efflux measurement period. The medium was then collected and the cells were harvested with 1 ml 1% SDS. *myo*-[³H]inositol ap-

pearance in the supernatant was measured by scintillation counting and expressed as the percentage of total *myo*-[³H]inositol in cells plus supernatant.

Statistics

Data are expressed as means \pm S.E.M. (*n* is the number of cell preparations from different animals on different days). Statistical analysis was performed with Student's *t* test. *P* < 0.05 was considered to be statistically significant.

RESULTS

Modulation of *myo*-inositol transport in liver macrophages by aniso-osmolarity

myo-Inositol was taken up by cultured rat Kupffer cells when incubated in normo-osmotic (305 m-osM) medium (Figure 1). *myo*-Inositol uptake was stimulated markedly when the cells were exposed to hyperosmotic (405 m-osM) medium (Figure 1). In contrast, exposure of Kupffer cells to hypo-osmotic (205 m-osM) medium lowered *myo*-inositol uptake (Figure 1). Induction of *myo*-inositol transport was strongly osmolarity dependent (Figure 2). *myo*-Inositol (10 μ M) uptake during a 2 h period in hyperosmotically pre-exposed Kupffer cells was not influenced by the simultaneous addition of 5 mM betaine or taurine (results not shown). This indicates a specific transport for *myo*-inositol that is distinct from betaine and taurine uptake.

At an extracellular concentration of 10 μ M, the intracellular *myo*-inositol concentration increased in hyperosmotic incubations to approx. 26 nmol/mg of protein within an 8 h period of exposure to *myo*-inositol (Figure 1). Given an intracellular water space of approx. 23.9 μ l/mg of protein (see the Materials and methods section), an intracellular/extracellular concentration gradient for *myo*-inositol of approx. 110 is created after hyperosmotic exposure. At a near-physiological extracellular *myo*-

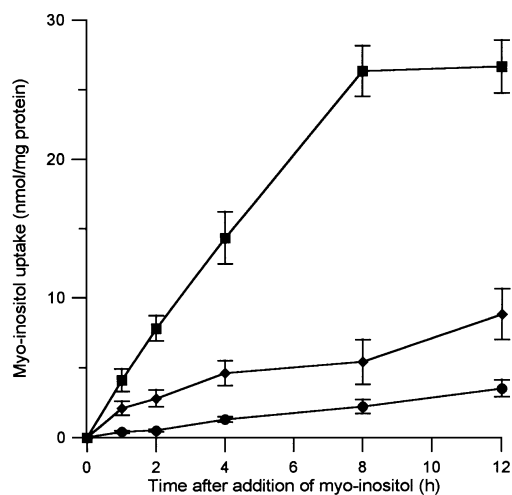


Figure 1 Effect of aniso-osmotic exposure on *myo*-inositol uptake by cultured rat liver macrophages (Kupffer cells)

Kupffer cells were kept in culture for 48 h. The incubation medium was then changed to hypo-osmotic (205 m-osM, ●), normo-osmotic (305 m-osM, ◆) or hyperosmotic (405 m-osM, ■) medium and the uptake of *myo*-[³H]inositol (10 μ M) was measured over the periods indicated as described in the Materials and methods section. Osmolarity changes were performed by the appropriate addition or removal of NaCl to/from the normo-osmotic (305 m-osM) control medium. Results are given as means \pm S.E.M. and are from three or four separate experiments for each condition.

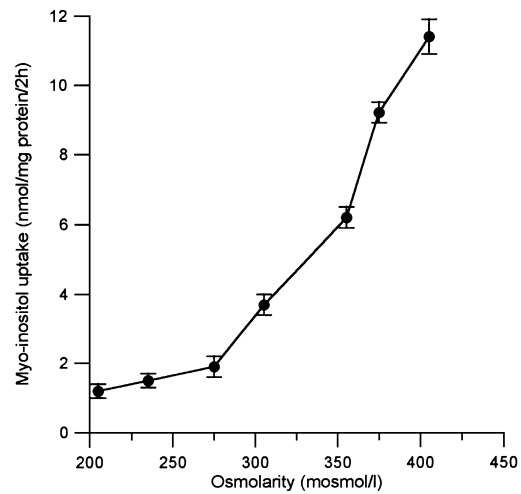


Figure 2 Osmolarity dependence of inducible *myo*-inositol uptake

Kupffer cells were kept in culture for 48 h. The incubation medium was then changed for 16 h to a medium with an osmolarity as indicated. Osmolarity changes were performed by the appropriate addition or removal of NaCl to/from the normo-osmotic (305 m-osM) control medium. After this 16 h preincubation period, the uptake of *myo*-[³H]inositol (10 μ M) over a 2 h period was measured as described in the Materials and methods section. Results are given as means \pm S.E.M. and are from three or four separate experiments for each condition.

Table 1 Intracellular *myo*-inositol concentrations in Kupffer cells

Kupffer cells were preincubated for 16 h in hypo-osmotic (205 m-osM), normo-osmotic (305 m-osM) or hyperosmotic medium (405 m-osM). Osmolarity changes were performed by the appropriate addition/removal of NaCl. The uptake of *myo*-[³H]inositol was then measured after 12 h in a *myo*-inositol (100 μ M)-containing medium with the same osmolarity. The intracellular *myo*-inositol concentrations were calculated on the basis of an intracellular water space of approx. 23.9 μ l/mg of protein (see the Materials and Methods section). Results are given as means \pm S.E.M. (*n* = 3).

Osmolarity (m-osM)	Uptake (nmol per 12 h per mg of protein)	Intracellular <i>myo</i> -inositol concentration (mM)
205	18 \pm 1	0.7
305	53 \pm 3	2.2
405	481 \pm 10	20.1

inositol concentration of 100 μ M, the intracellular *myo*-inositol concentration is calculated to be approx. 20 mM under hyperosmolarity (Table 1), indicating that *myo*-inositol accumulation in response to hyperosmolarity contributes significantly to intracellular osmolarity.

In line with an increased *myo*-inositol transport, hyperosmotic (405 m-osM) exposure of Kupffer cells led to a strong and time-dependent increase in mRNA levels for SMIT (Figure 3). Maximal SMIT mRNA levels were found after 6–12 h of hyperosmotic exposure. GAPDH mRNA levels were chosen as standard, because this mRNA was shown to be uninfluenced by aniso-osmolarity [28]. The hyperosmolarity-induced increase in SMIT mRNA levels was diminished by 72 \pm 4% or by 52 \pm 6% in the presence of 5 mM *myo*-inositol or betaine respectively, but not in the presence of taurine after 12 h (*n* = 3). Here the SMIT mRNA levels were 99 \pm 8% of the control value observed in absence of taurine.

To test whether the use of *myo*-inositol is Kupffer cell-specific, the uptake of osmolytes by RAW 264.7 macrophages was measured. As shown previously, RAW 264.7 cells express an

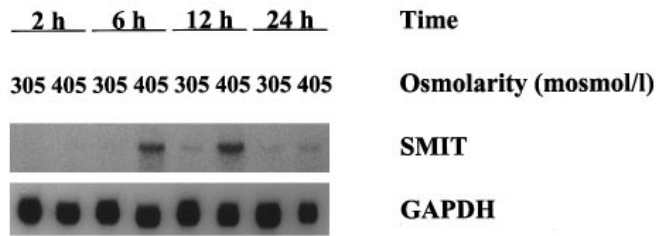


Figure 3 Time-dependent induction of SMIT mRNA levels in Kupffer cells during hyperosmolarity

Kupffer cells were exposed in normo-osmotic (305 m-osM) or hyperosmotic (405 m-osM) media for the periods indicated and mRNA levels for SMIT and GAPDH were determined by Northern blot analysis (7 μ g of total RNA per lane). These results are representative of three separate experiments.

Table 2 Organic osmolyte transport in Kupffer cells and RAW 264.7 cells

Kupffer cells or RAW 264.7 cells were preincubated for 16 h in hypo-osmotic (205 m-osM), normo-osmotic (305 m-osM) or hyperosmotic medium (405 m-osM). Osmolarity changes were performed by the appropriate addition/removal of NaCl. The uptake of 100 μ M [3 H]taurine, [14 C]betaine or *myo*-[3 H]inositol was then measured for 2 h in medium with the same osmolarity. Results are given as means \pm S.E.M. ($n = 3-5$).

	Uptake (nmol per 2 h per mg of protein)			
	Osmolarity (m-osM) ...	205	305	405
Kupffer cells				
Taurine		29 \pm 4	79 \pm 11	113 \pm 9
Betaine		4 \pm 1	15 \pm 1	156 \pm 13
<i>myo</i> -Inositol		4 \pm 1	5 \pm 1	94 \pm 9
RAW 264.7 cells				
Taurine		55 \pm 1	163 \pm 19	183 \pm 8
Betaine		11 \pm 1	78 \pm 4	168 \pm 9
<i>myo</i> -Inositol		3 \pm 1	5 \pm 1	9 \pm 1

osmosensitive betaine transporter, whose activity and BGT-1 mRNA levels are up-regulated after hyperosmotic exposure and down-regulated during hypo-osmolarity [17]. Kupffer cells and RAW 264.7 cells were preincubated for 16 h in hypo-osmotic (205 m-osM), normo-osmotic (305 m-osM) or hyperosmotic (405 m-osM) medium and the uptake of 100 μ M [3 H]taurine, [14 C]betaine or *myo*-[3 H]inositol was then measured over a 2 h period in the respective medium. Values of uptake over 2 h were chosen to minimize the potential effects of the osmolytes on a substrate-induced down-regulation of the transporters. It should be noted that owing to the slow accumulation of osmolytes into the cells (see Figure 1 in [16], and Figure 1 in [17]), such 2 h uptake values do not reflect intracellular steady-state osmolyte levels but rather a rough approximation of the initial uptake rates. In contrast with betaine transport, *myo*-inositol uptake was low in RAW 264.7 cells, and there was only slight stimulation of *myo*-inositol uptake by hyperosmolarity (Table 2). Further, taurine was strongly taken up by RAW 264.7 cells when incubated in normo-osmotic (305 m-osM) medium (Table 2). Taurine uptake was 15–20-fold higher than *myo*-inositol uptake and there was also slight further stimulation of taurine uptake by hyperosmolarity (Table 2). Exposure of RAW 264.7 cells to hypo-osmotic (205 m-osM) medium for 16 h markedly lowered the taurine content (Table 2). TAUT mRNA levels were also dependent on ambient osmolarity, but under normo-osmotic

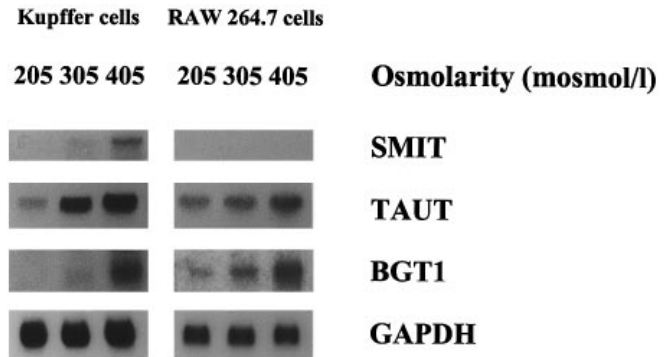


Figure 4 Organic osmolyte transporter mRNA levels in Kupffer cells and RAW 264.7 cells

Kupffer or RAW 264.7 cells were exposed in hypo-osmotic (205 m-osM), normo-osmotic (305 m-osM) or hyperosmotic (405 m-osM) media for 12 h and mRNA levels for SMIT, TAUT, BGT1 and GAPDH were determined by Northern blot analysis (7 μ g of total RNA per lane). These results are representative of three separate experiments.

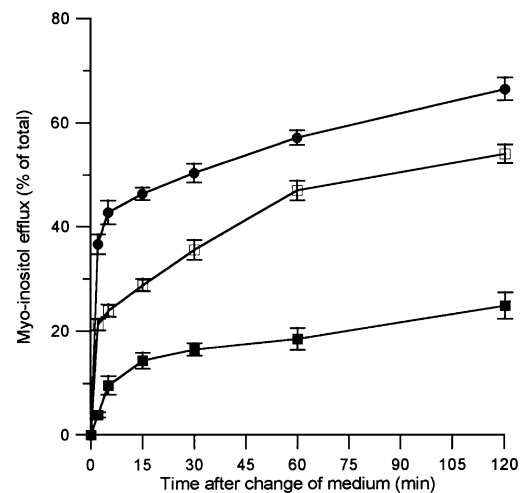


Figure 5 *myo*-Inositol efflux from rat Kupffer cells after hypo-osmotic exposure

Kupffer cells were preincubated for 16 h in hyperosmotic medium (405 m-osM, prepared by the addition of 50 mM NaCl) to induce *myo*-inositol transport activity. Kupffer cells were then allowed to accumulate *myo*-[3 H]inositol (added at a concentration of 10 μ M) for 4 h. The cells were then quickly washed three times and thereafter exposed to *myo*-inositol-free hyperosmotic (405 m-osM, \square), hyperosmotic (405 m-osM, \bullet) containing latex (0.025%, 1 μ m in diameter) or hypo-osmotic (205 m-osM, \blacktriangle) Krebs–Henseleit buffer. The appearance of *myo*-[3 H]inositol in the supernatant was measured and expressed as the percentage of total *myo*-[3 H]inositol in cells plus supernatant. Results are given as means \pm S.E.M. and are from three or four separate experiments for each condition.

conditions TAUT mRNA levels were already high (Figure 4). In line with the low *myo*-inositol transport (Table 2), mRNA for SMIT was not detectable in RAW 264.7 cells and there was no induction by hyperosmolarity (Figure 4).

Studies on *myo*-inositol efflux from Kupffer cells

Cultured Kupffer cells were kept for 12 h in hyperosmotic medium (405 m-osM) to induce *myo*-inositol transport. They were then preloaded with *myo*-inositol by incubating the cells in the same medium supplemented with *myo*-[3 H]inositol (10 μ M) for 4 h and the cells were washed three times in *myo*-inositol-free

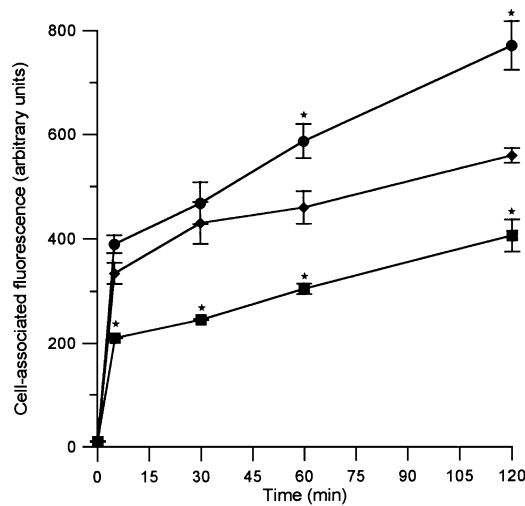


Figure 6 Effect of aniso-osmolarity on phagocytosis of latex particles by cultured rat Kupffer cells

Kupffer cells were incubated for 20 h in hypo-osmotic (205 m-osM, ●), normo-osmotic (305 m-osM, ◆) or hyperosmotic (405 m-osM, ■) medium. Osmolarity was altered by varying the NaCl concentration in the medium. Thereafter, 0.025% fluorescein-coupled latex particles (1 μm diameter) were added to the media for 5, 30, 60 or 120 min. Phagocytosis was measured as cell-associated fluorescence after latex addition and fluorescence was expressed as arbitrary units. Results are given as means ± S.E.M. and are from three separate experiments for each condition. * Statistically significant difference ($P < 0.05$) from the normo-osmotic (305 m-osM) incubations.

hyperosmotic medium. The cells were then exposed to Krebs–Henseleit test medium and the release of *myo*-[³H]inositol from the cells was monitored. When the test medium was hyperosmotic, the cells released only approx. 20% of the intracellularly contained *myo*-inositol within 2 h (Figure 5). When the test medium was hypo-osmotic (205 m-osM), there was a rapid efflux of *myo*-inositol from the cells and approx. 40% of intracellular *myo*-inositol was already released within 5 min (Figure 5). This *myo*-inositol efflux was inhibited by only 12 ± 3% ($n = 3$) in the presence of the anion-exchanger inhibitor DIDS (0.1 mmol). The cell viability tested by LDH release at the end of the 2 h incubations was not significantly different under the various test conditions, and LDH release was 6–8 units/l. These results indicate that *myo*-inositol acts as an osmolyte in Kupffer cells and is released in response to hypo-osmotic cell swelling.

Studies on the phagocytosis of latex particles in Kupffer cells

As shown previously, Kupffer cells in culture avidly phagocytose latex particles [22]. Phagocytosis of Kupffer cells was measured as described in the Materials and methods section after exposure of the cells to fluorescein-coupled latex particles (0.025%, 1 μm in diameter) for the periods indicated. When Kupffer cells were exposed to aniso-osmotic media for 20 h before the addition of latex particles, hyperosmotic (405 m-osM) exposure decreased phagocytosis by approx. 30% compared with the normo-osmotic control, whereas phagocytosis was stimulated by approx. 38% during hypo-osmolarity (205 m-osM) (Figure 6 and Table 3). The addition of 10 or 100 μM *myo*-inositol 4 h before latex addition counteracted the inhibition of phagocytosis by hyperosmolarity (Table 3). The effects of aniso-osmolarity and *myo*-inositol on phagocytosis were not explained by differences in cell viability, which was tested by LDH release at the end of the incubations and was 7–10 units/l under all conditions.

Table 3 Effect of hyperosmolarity and *myo*-inositol on phagocytosis of latex particles by cultured rat Kupffer cells over a 1 h period

Kupffer cells were exposed for 20 h to normo-osmotic (305 m-osM) or hyperosmotic (405 m-osM) medium. The osmolarity change was performed by appropriate change in the NaCl concentration. Thereafter, 0.025% fluorescein-coupled latex particles (1 μm in diameter) were added to the medium. The influence of *myo*-inositol on phagocytotic activity was examined by the addition of 10 or 100 μM *myo*-inositol 4 h before the addition of latex particles. Phagocytosis was measured as cell-associated fluorescence 1 h after latex addition; fluorescence is expressed as arbitrary units (AU). Results are given as means ± S.E.M. for three separate experiments for each condition. Statistical significance levels: * $P < 0.05$ compared with the normo-osmotic (305 m-osM) incubation; † $P < 0.05$ compared with the hyperosmotic (405 m-osM) condition without *myo*-inositol.

Conditions	Cell-associated fluorescence	
	(AU)	(%)
305 m-osM	458 ± 32†	100
405 m-osM	303 ± 31*	66
405 m-osM + 10 μM <i>myo</i> -inositol	374 ± 6*†	82
405 m-osM + 100 μM <i>myo</i> -inositol	415 ± 3†	91

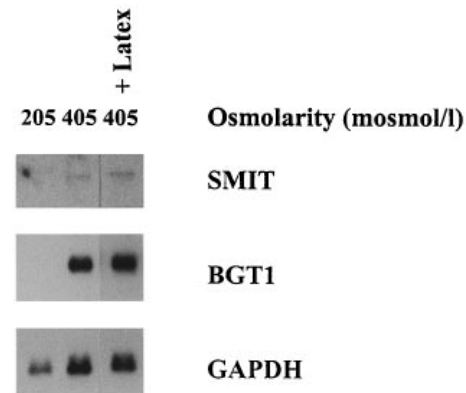


Figure 7 Effect of latex particles on the hyperosmolarity-induced increase in SMIT and BGT-1 mRNA levels in rat Kupffer cells

Cultured Kupffer cells were exposed for 12 h to hyperosmotic (405 m-osM) medium in the presence or absence of 0.0125% latex particles or to hypo-osmotic (205 m-osM) medium as described in the Materials and methods section. The osmolarity changes were performed by appropriate changes in the NaCl concentration. The cells were then harvested for RNA isolation and subjected to Northern blot analysis for SMIT, BGT-1 and GAPDH (7 μg of total RNA per lane). These results are representative of three separate experiments.

When Kupffer cells were preloaded with *myo*-inositol, stimulation of phagocytosis by the addition of latex particles stimulated *myo*-inositol efflux from the cells (Figure 5). The kinetics of the time course of phagocytosis during hyperosmolarity was similar to that of phagocytosis-induced *myo*-inositol efflux (compare Figures 5 and 6). Whereas the stimulation of phagocytosis induced a rapid *myo*-inositol efflux from the cells, it was without effect on SMIT and BGT1 mRNA levels (Figure 7).

DISCUSSION

myo-Inositol is an important metabolite in the synthesis of phosphoinositides, and, as an osmolyte, *myo*-inositol contributes to the regulation of cell volume in several cell types, including kidney, lens epithelia, urinary bladder, astrocytes and endothelial cells [8–11,29]. In general, in most tissues the intracellular concentration of *myo*-inositol is severalfold higher than the

circulating levels [30,31]. These high concentrations are maintained by uptake mechanisms or by direct conversion of glucose to inositol [32–36]. The *myo*-inositol uptake into isolated rat hepatocytes occurs by a carrier-mediated process that is different from that for glucose [37]. mRNA for the osmosensitive *myo*-inositol-transport system SMIT was not detectable in rat hepatocytes and H4IIE hepatoma cells [19]. In line with this, *myo*-inositol uptake by H4IIE cells was low and not stimulated by hyperosmolarity [19].

The results of the present study demonstrate that *myo*-inositol is an osmolyte in rat liver macrophages (Kupffer cells) but not in RAW 264.7 mouse macrophages. The use of *myo*-inositol as an osmolyte by Kupffer cells is evidenced by the osmolarity-dependent intracellular accumulation of *myo*-inositol inside cells (Figures 1 and 2), the osmosensitivity of SMIT mRNA levels (Figures 3 and 4) and the stimulation of *myo*-inositol efflux from the cells in response to hypo-osmotic cell swelling (Figure 5). The physiological *myo*-inositol concentration in blood was reported to be 40–80 μM [33]. In the presence of a near-physiological *myo*-inositol concentration of 100 μM , the intracellular *myo*-inositol concentration in Kupffer cells is calculated to increase from 2 to 20 mM in response to hyperosmolarity, suggesting that *myo*-inositol is, like betaine [16] and taurine [18], a significant osmolyte in Kupffer cells. In contrast with Kupffer cells, *myo*-inositol transport was low in RAW 264.7 cells (Table 2); the mRNA for SMIT was not detectable and was not induced by hyperosmolarity (Figure 4). These results suggest that *myo*-inositol has little or no role as an osmolyte in these cells. It is unclear whether this difference in *myo*-inositol uptake between rat Kupffer cells and RAW 264.7 mouse macrophages resides in the malignant phenotype of the latter cell type or whether it is specific for liver macrophages. It remains to be established whether Kupffer cells or RAW 264.7 cells are able to synthesize *myo*-inositol from glucose. In contrast, betaine and taurine are used as osmolytes in both Kupffer cells [16,18] and RAW 264.7 cells [17] (Figure 4 and Table 2).

In line with recent findings on betaine transport [16], *myo*-inositol efflux from Kupffer cells in response to hypo-osmolarity was much faster than uptake during hyperosmolarity. There is general agreement that osmolyte efflux occurs via routes distinct from uptake [38]. In C6 glioma cells and Madin–Darby canine kidney cells, volume-sensitive anion channels mediate the passive loss of certain organic osmolytes from the cells [38]; however, in contrast with C6 glioma cells, in which *myo*-inositol efflux occurs slowly after hypo-osmolarity [39], the *myo*-inositol release from Kupffer cells was fast. In Kupffer cells, the swelling-activated *myo*-inositol efflux was only slightly inhibited by the anion-exchanger inhibitor DIDS, whereas hypo-osmotic taurine efflux was strongly DIDS-sensitive [18]. The route of *myo*-inositol efflux, however, remains to be established.

The rate of uptake of *myo*-inositol was not influenced by the simultaneous addition of taurine or betaine for 2 h under the hyperosmotic condition, suggestive of distinct uptake routes for these osmolytes. In contrast, the hyperosmolarity-induced induction of SMIT mRNA was in part prevented by preincubation with *myo*-inositol and betaine for 12 h, but not by taurine. This might be explained by differential effects of these osmolytes on cell volume when the cells are challenged with hyperosmotic media. Whereas hyperosmotic exposure is followed by a marked increase in the cellular betaine and *myo*-inositol contents (Table 2), taurine levels are already high in normo-osmotic incubations and increase only mildly after hyperosmotic exposure. Thus a higher potency of *myo*-inositol and betaine than of taurine to counteract hyperosmolarity-induced cell shrinkage might explain the suppression of SMIT mRNA induction by betaine and *myo*-

inositol, but not by taurine. Another explanation could be that Kupffer cells are heterogeneous with respect to the use of different osmolytes.

Myo-inositol is apparently engaged in the maintenance of cell volume during phagocytosis. The phagocytosis of latex particles by Kupffer cells was inhibited by hyperosmotic exposure (Figure 6), and this inhibition can be overcome by *myo*-inositol (Table 3). However, like betaine [16] and taurine [18], *myo*-inositol is also released from Kupffer cells during phagocytosis (Figure 5). The time courses of phagocytosis on the one hand and of phagocytosis-induced *myo*-inositol efflux on the other were similar (Figures 5 and 6). However, phagocytosis did not alter TAUT [18], SMIT or BGT-1 mRNA levels (Figure 7). These findings suggest that phagocytosis augments an osmolyte efflux pathway from the cells, as it is observed in response to hypo-osmotic cell swelling, but might have no effect on unidirectional concentrative osmolyte uptake via TAUT, BGT1 or SMIT. Because ingestion of material susceptible to phagocytosis by Kupffer cells should be accompanied by an increase of cellular volume, these findings could suggest that osmolyte efflux might be used in these cells for compensation of a phagocytosis-induced cell volume increase. Thus *myo*-inositol might contribute to isotonic cell volume homeostasis during phagocytosis in Kupffer cells. Kupffer cells are located in the liver sinusoids and gross changes of their cell volume during phagocytosis should affect sinusoidal resistance and liver blood flow unless potent volume regulatory mechanisms are present. Further studies are required to clarify the question whether the different osmolytes in Kupffer cells, i.e. betaine, taurine and *myo*-inositol fulfil different functions.

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