Modulation of phagocytosis by anisoosmolarity and betaine in rat liver macrophages (Kupffer cells) and RAW 264.7 mouse macrophages

Ulrich Warskulat, Fan Zhang, Dieter Häussinger*

Medizinische Universitätsklinik, Klinik für Gastroenterologie, Hepatologie und Infektiologie, Heinrich-Heine-University, Moorenstrasse 5, D-40225 Düsseldorf, Germany

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Abstract Hypoosmotic exposure (205 mosmol/l) of rat liver macrophages (Kupffer cells) for 12 h stimulated phagocytosis of latex particles by about 20%, whereas hyperosmotic exposure (405 mosmol/l) resulted in 30–40% inhibition. Inhibition of phagocytosis by hyperosmolarity was fully prevented in the presence of betaine, which acts as an osmolyte in liver macrophages. When hyperosmotically exposed Kupffer cells were preloaded with betaine, induction of phagocytosis by addition of latex particles led to the stimulation of betaine efflux from the cells. Stimulation of phagocytosis also inhibited the hyperosmolarity-induced cumulative uptake of betaine into Kupffer cells, but did not prevent the hyperosmolarity-induced increase in BGT1-mRNA levels. Whereas these findings suggest an involvement of cell volume and betaine in the regulation of phagocytosis in Kupffer cells, betaine transport was not affected upon induction of phagocytosis in RAW 264.7 mouse macrophages. The findings are compatible with a role of betaine in maintaining cell volume homeostasis during phagocytosis in Kupffer cells, but not in RAW 264.7 mouse macrophages. This may be relevant for the maintenance of liver hemodynamics, since volume changes of liver macrophages following ingestion of phagocytozable material might otherwise impair sinusoidal perfusion.

Key words: Kupffer cell; RAW 264 mouse macrophage; Betaine; Osmolyte; Hyperosmolarity; Cell volume; Osmolarity; Phagocytosis; Latex; Macrophage; BGT-1

1. Introduction

Liver macrophages (Kupffer cells) belong to the mononuclear phagocyte system and play an important role in the body's defense machinery (for review see [1]). Upon stimulation with endotoxin these cells produce a variety of cytokines, lipid mediators and radicals. Recent studies have indicated that endotoxin enhances the expression of inducible cyclooxygenase-2 (Cox-2) [2–6], which results in the increased formation of prostanoids by Kupffer cells and other macrophages [3,7–9]. Interestingly, lipopolysaccharide (LPS)-induced prostaglandin E_2, D_2 and thromboxane B_2 formation and Cox-2 expression are stimulated up to 10-fold when ambient osmolarity increases from 300 to 350 mosmol/l [3]. This remarkably sensitive and potent osmoregulation suggested that cell volume homeostasis is critical for Kupffer cell function. Subsequent studies demonstrated that Kupffer cells [10] and RAW 264.7 mouse macrophages [11] use betaine as an organic osmolyte and that mRNA levels coding for the betaine transporter BGT-1 are strongly induced by hyperosmolarity in rat Kupffer cells and mouse RAW 264.7 macrophages [10,11]. Organic osmolytes are compounds which are specifically accumulated or released by the cells in response to hyperosmotic cell shrinkage or hypoosmotic cell swelling, respectively, in order to maintain cell volume homeostasis. Osmolytes need to be non-perturbing solutes that do not interfere with protein function even when occurring in high intracellular concentrations (for reviews see [12–14]). The functional significance of hyperosmolarity-induced betaine accumulation in Kupffer cells is suggested by the fact that betaine was shown to suppress the hyperosmolarity-induced stimulation of prostaglandin formation and Cox-2 expression [10]. Thus, osmolyte availability and BGT-1 expression are another site of regulation of Kupffer cell function. The present study was undertaken in order to gain insight into the role of cell volume and of the osmolyte betaine in the regulation of phagocytosis in rat Kupffer cells.

2. Materials and methods

2.1. Materials

Polybead fluorescent microspheres (2.5% solids latex, 1 μm diameter) were obtained from Polysciences Ltd. (St. Goar, Germany). RPMI 1640 medium (without phenol red) and fetal calf serum (FCS) for culture of Kupffer cells were from Biochrom (Berlin, Germany), DME medium and FCS for culture of RAW 264.7 cells were from Gibco (Eggenstein, Germany). RNeasy total RNA kit was from Qiagen (Hilden, Germany) and oligonucleotide-labelling kit was from Pharmacia (Freiburg, Germany). Sodium dodecyl sulfate was from Fluka (Karlsruhe, Germany). Nycodenz was obtained from Nycomed (Oslo, Norway). Hybond-N nylon membranes were purchased from Amersham Buchler (Braunschweig, Germany). [α-^{32}P]dCTP (3000 Ci/mmol) was from ICN (Meckenheim, Germany) and [^{14}C]betaine (48.1 mCi/mmol) was from New England Nuclear DuPont (Bad Homburg, Germany). Betaine and lipopolysaccharide (LPS) were from Sigma (Deisenhofen, Germany). A plasmid containing full-length BGT-1 cDNA [15] was kindly provided by Dr. H. Moo Kwon (Division of Nephrology, The Johns Hopkins University, Baltimore, USA). The 1.0 kb cDNA fragment for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was used for standardization, was from Clontech (Palo Alto, USA).

2.2. Isolation and culture of Kupffer cells

Rat Kupffer cells were prepared by collagenase-pronase perfusion and separated by a single Nycodenz gradient and centrifugal elution as described previously [3,16]. Cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS) for 48 h. The experiments were performed during the following 24 h using Krebs-Henseleit hydrogen carbonate buffer (pH 7.4) containing 10 mmol/l 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. Viability of the incubations was routinely tested based on lactate dehydrogenase (LDH) release at the end of the incubations.

*Corresponding author. Fax: (49) (211) 8118838.

Abbreviations: LPS, lipopolysaccharide; FCS, fetal calf serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; BGT-1, betaine gamma-aminobutyric acid transporter

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2.3. Culture of RAW 264.7 mouse macrophages

RAW 264.7 mouse macrophages (A.T.C.C. TIB 71) were grown to near confluency in DME medium, 37°C, 5% CO_2, pH 7.4, supplemented with 10% FCS in Cluster 6 dishes (Costar, Cambridge, USA). The osmolarity was varied by changing the NaCl concentration in the medium. For identification of BGT-1 mRNA levels, cells had grown for 12 h in serum-free DME medium under various osmotic test conditions in the presence or absence of latex particles (0.0125%).

2.4. Determination of phagocytic activity

The phagocytotic activity of Kupffer cells and RAW 264.7 mouse macrophages, i.e. the ability to ingest fluorescent latex particles, was assessed by measuring the cell-associated fluorescence [17]. Briefly, 10^6 Kupffer cells or RAW 264.7 mouse macrophages were plated on 24-well culture plates and were incubated with RPMI 1640 medium for 48 h. Then the non-adherent cells were removed and the medium was replaced by Krebs-Henseleit buffer containing 0.025% solid fluorescent latex particles (1 μm diameter). After 1 h in a 5% CO_2-air atmosphere at 37°C, the cells were washed four times with Krebs-Henseleit buffer. Kupffer or RAW 264.7 cells were removed from each well using a rubber policeman and the cell suspension was used for fluorescence measurements with excitation at 436 nm and emission above 500 nm. Control plates were used for microscopy. The percentage of cells phagocytosing latex was usually greater than 95%. Phagocytosis under test conditions was expressed as the percentage of fluorescence intensity found when compared to that found under normoosmotic control conditions.

2.5. Uptake and efflux of betaine

The uptake of betaine by control and latex-treated Kupffer cells was assessed as described previously [10,11]. In brief, Kupffer cells were incubated in 0.5 ml of Krebs-Henseleit buffer with [^{14}C]betaine (10 μmol/l, 0.5 μCi/ml) for 2 h at 37°C. Then, the cells were washed four times with ice-cold stop solution (10 mmol/l Tris-HEPES pH 7.4, 300 mmol/l mannitol and 300 mmol/l NaCl), and dried in air at room temperature. Thereafter, the cells were harvested with 1 ml 1% sodium dodecyl sulfate (SDS) and aliquots were taken for liquid scintillation counting and protein determination (Bio-Rad protein assay, Bio-Rad Labs., Hercules, USA) with bovine serum albumin as standard. As shown previously by HPLC analysis, betaine was not metabolized by Kupffer cells [10] and the radioactivity recovered was assumed to reflect betaine, the intracellular amount of which was calculated on the basis of the specific radioactivity of added betaine.

Betaine efflux was measured as described previously [10,11]. In brief, Kupffer cells were preincubated for 12 h in hyperosmotic bicarbonate-buffered Krebs-Henseleit saline (405 mosmol/l, being prepared by addition of 50 mmol/l NaCl) in Cluster 24 dishes (Costar, Cambridge, USA), in order to induce betaine transport activity. Then the Kupffer cells were incubated in hyperosmotic Krebs-Henseleit buffer (405 mosmol/l) containing [^{14}C]betaine (10 μmol/l, 0.5 μCi/ml) in order to load the cells with radioisotope as described previously [10,11]. The RAW 264.7 cells were incubated in serum-free hyperosmotic DME medium (405 mosmol/l) containing [^{14}C]betaine (10 μmol/l, 0.5 μCi/ml). After a loading period of 2 h at 37°C, the cells were quickly rinsed three times with hyperosmotic incubation buffer, containing 10 mmol/l Tris-HEPES pH 7.4, 5 mmol/l KCl, 2 mmol/l $CaCl_2$, 1.2 mmol/l $MgCl_2$ and 193 mmol/l NaCl. Then the Kupffer and RAW 264.7 cells were incubated for 30–120 min in betaine-free hyperosmotic (405 mosmol/l), hyperosmotic (405 mosmol/l) containing latex (0.025%, 1 μm diameter) or hypoosmotic (205 mosmol/l) incubation buffer, being prepared by removal of 100 mmol/l NaCl from the hyperosmotic incubation buffer described above. At the end of the incubation, the medium was collected and cells were harvested with 1 ml 1% SDS. The appearance of ^{14}C radioactivity in the incubation medium and radioactivity retained in the cell extracts was measured by scintillation counting. [^{14}C]Betaine appearance in the supernatant was measured and expressed as the percentage of total [^{14}C]betaine (contained in cells plus supernatant).

2.6. Northern blot analysis

Total RNA from near-confluent culture plates of Kupffer cells or RAW 264.7 cells was isolated by using the RNeasy Total RNA Kit (Qiagen, Hilden, Germany). RNA samples were electrophoresed in 0.8% agarose/3% formaldehyde and then blotted onto Hybond-N nylon membranes with 20×SSC (3 mol/l NaCl, 0.3 mol/l sodium citrate). After brief rinsing with water and UV-crosslinking (Hoefer UV-crosslinker 500, Hoefer, San Francisco, USA), the membranes were observed under UV illumination to determine RNA integrity and location of the 28S and 18S rRNA bands. Blots were then subjected to 3 h prehybridization at 43°C in 50% deionized formamide, in sodium phosphate buffer (0.25 mol/l, pH 7.2), containing 0.25 mol/l NaCl, 1 mmol/l EDTA, 100 μg/ml salmon sperm DNA and 7% SDS. Hybridization was carried out in the same solution with approx. 10^6 cpm/ml [α-^{32}P]dCTP-labeled random-primed BGT1 or GAPDH cDNA probes. Membranes were washed three times in 2×SSC/0.1% SDS for 10 min, twice in sodium phosphate buffer (25 mmol/l, pH 7.2)/EDTA (1 mmol/l)/0.1% SDS and twice in sodium phosphate buffer (25 mmol/l, pH 7.2)/EDTA (1 mmol/l)/1% SDS. Blots were then exposed to Kodak AR X-omat film at −70°C with intensifying screens. Suitably exposed autoradiograms were then analysed by densitometry scanning (PDI, New York, USA).

2.7. Statistics

Values are expressed as means ± S.E.M. (n = number of cell preparations). Statistical analysis was performed using Student's t-test. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effect of anisoosmolarity on the phagocytosis of latex particles in cultured rat liver macrophages (Kupffer cells)

Phagocytosis of cultured Kupffer cells was measured as described in Section 2 following exposure of the cells to fluorescein-coupled latex particles (1 μm diameter) for 1 h. During the process of phagocytosis, latex particles accumulate inside the cells (Fig. 1A). The amount of fluorescence accumulating inside the cells within 1 h in normoosmotic incubations was set to 100%. When Kupffer cells were exposed to anisoosmotic media for 12 h before addition of latex, hypoosmotic (205 mosmol/l) exposure increased phagocytosis by about 20%, whereas hyperosmolarity (405 mosmol/l) inhibited by about 35% (Table 1). Thus, lowering the osmolarity from 405 to 205 mosmol/l almost doubled the phagocytotic activity of Kupffer cells. Interestingly, betaine counteracted in a dose-dependent manner the inhibition of phagocytosis by hyperosmolarity (Table 1). As also shown in Table 1, the effects of anisoosmolarity on phagocytosis were not explained by differences in cell viability (LDH leakage).

Inhibition of phagocytosis by hyperosmolarity was also observed after immediate exposure to hyperosmolarity; i.e. when the hyperosmotic stress was instituted together with latex addition. When phagocytosis under normoosmotic control conditions was set to 100%, hyperosmolarity (405 mosmol/l) due to addition of NaCl (50 mmol/l), glucose (100 mmol/l) or raffinose (100 mmol/l) lowered phagocytosis to 73 ± 6, 55 ± 6 or 48 ± 6% (n = 4–5), respectively. Also, under these conditions, addition of betaine (10 mmol/l) increased phagocytosis in normoosmotic controls from 100 ± 2 to 119 ± 4% (n = 4), from 77 ± 4 to 104 ± 6% (n = 4) in hyperosmotic media (405 mosmol/l due to addition of NaCl) and from 61 ± 5 to 81 ± 8% (n = 4) in hyperosmotic media due to addition of glucose (100 mmol/l).

3.2. Phagocytosis and betaine transport in Kupffer cells

As shown recently, exposure of cultivated Kupffer cells to hyperosmotic media leads to the induction of a cumulative betaine transporter as shown by the increase in BGT-1 mRNA levels and stimulation of betaine uptake by the cells [10]. In order to study the effect of phagocytosis on betaine transport, Kupffer cells were preexposed to hyperosmotic

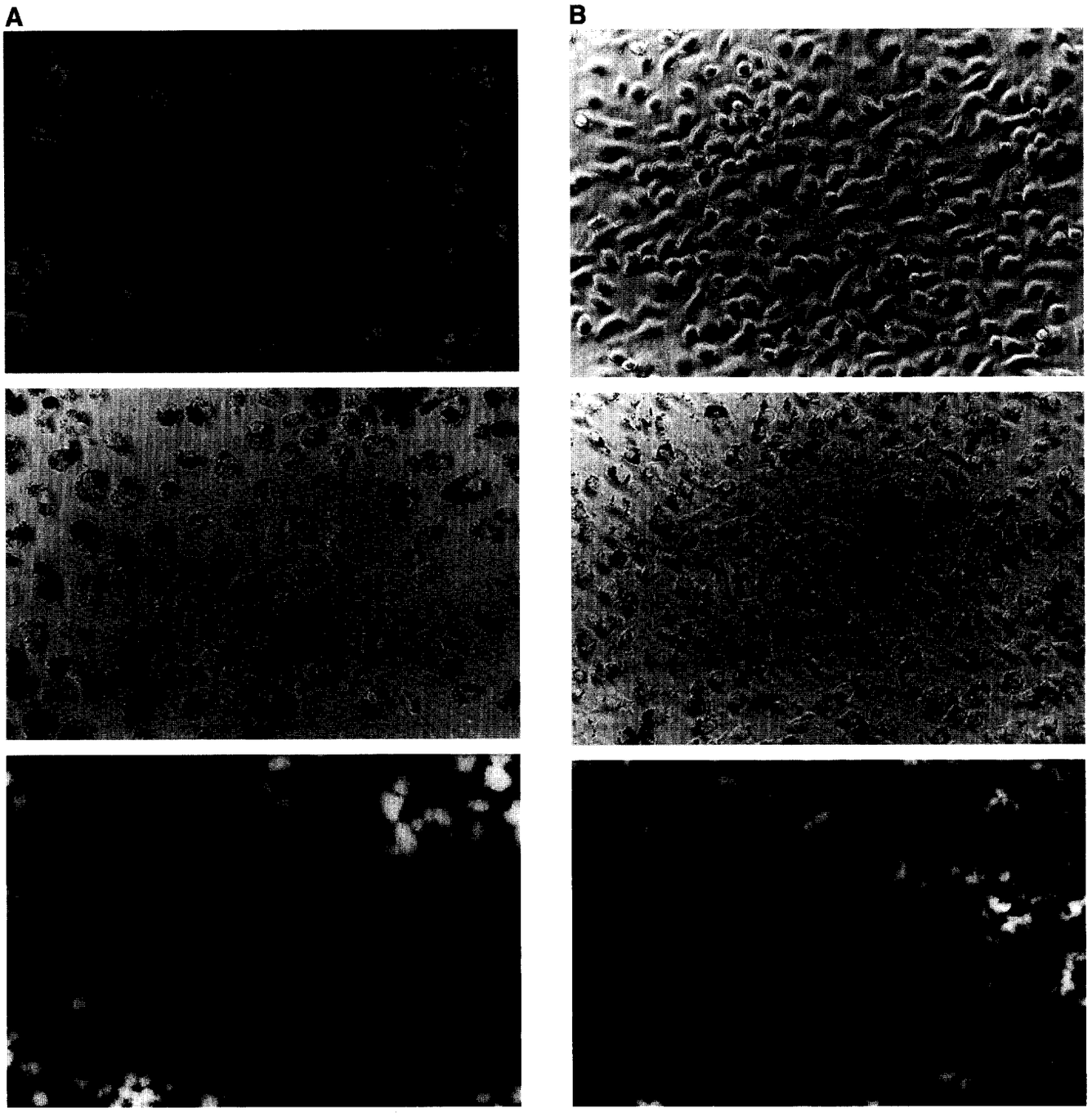

Fig. 1. Phagocytosis of latex particles by rat Kupffer cells (A) and RAW 264.7 mouse macrophages (B). Top: light microscopy of native Kupffer and RAW 264.7 cells. Middle: light microscopy of cells following exposure to 0.025% latex particles for 1 h. Bottom: fluorescence microscopy of phagocytozing Kupffer and RAW 264.7 cells.

media (405 mosmol/l) for 12 h in order to induce betaine transport. Then the cells were preloaded with [^{14}C]betaine (10 μmol/l) for 2 h in the same medium. Thereafter, the medium was changed to betaine-free media in order to monitor betaine efflux from the cells. When this medium was again hyperosmotic (405 mosmol/l), the cells released 20–30% of the intracellular betaine during the next 1–2 h. However, when phagocytosis was stimulated simultaneously by the addition of latex particles, betaine efflux was significantly increased and about 50% of cellular betaine was released after 2 h of phagocytosis (Fig. 2). As for controls, a hypoosmotic (205 mosmol/l) challenge induced the release of about 60% of the betaine contained inside the cells (Fig. 2), in line with the role of betaine as an organic osmolyte in these cells [10]. These findings show that not only hypoosmotic stress but also induction of phagocytosis stimulates the efflux of betaine from liver macrophages. The difference in the time course of betaine efflux under both conditions may be explained by the

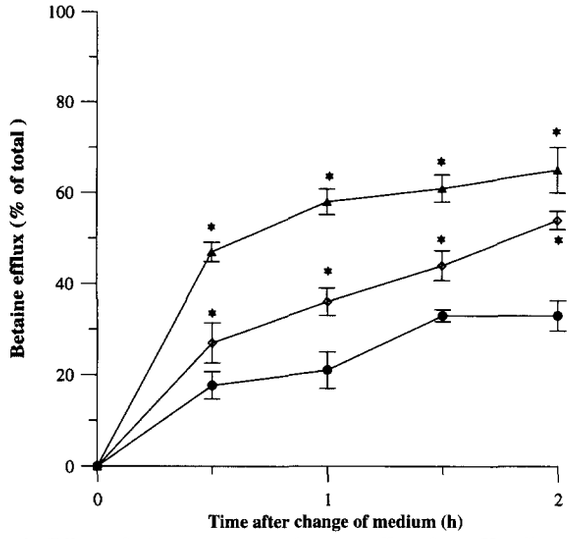

Fig. 2. Effect of phagocytosis on betaine efflux from Kupffer cells. Rat Kupffer cells were cultured for 48 h and subsequently exposed for 12 h to hyperosmotic (405 mosmol/l) medium, in order to induce cumulative betaine transport. The cells were then preloaded with [^{14}C]betaine (10 μmol/l and 0.5 μCi/ml) for 2 h in hyperosmotic medium. Thereafter, extracellular betaine was removed and the release of cellular betaine (expressed as % of total betaine contained in the cells) was monitored following addition of hyperosmotic (405 mosmol/l) medium without (●) or with (◇) 0.025% latex particles or following addition of hypoosmotic (205 mosmol/l) medium (▲). Data are given as means ± S.E.M. ($n = 4$). *Significantly different from the hyperosmotic (405 mosmol/l) condition without latex ($P < 0.05$).

different kinetics of cell volume increase following hypoosmotic exposure (almost immediate) and phagocytosis (slowly increasing with the amount of ingested material), respectively.

Stimulation of phagocytosis not only stimulates betaine efflux, but also inhibits betaine (10 μmol/l) uptake by Kupffer cells (Table 2). Betaine uptake was 19.7 ± 0.8 nmol/mg protein per 2 h in cells exposed for 14 h to hyperosmotic medium and, as shown previously [10], betaine uptake is linear over this 2 h period. Betaine uptake, however, decreased significantly to 13.3 ± 1.8 nmol/mg protein per 2 h when latex particles were added during the last 2 h of hyperosmotic exposure and even to 7.7 ± 1.2 nmol/mg protein per 2 h when latex was present throughout the 14th period of hyperosmolarity (Table 2). As shown in Fig. 3A, latex had no effect on the hyperosmolarity-induced induction of BGT-1 mRNA levels.

3.3. *Phagocytosis and betaine transport in RAW 264.7 mouse macrophages*

In order to test whether the interaction between betaine transport and phagocytosis is Kupffer cell-specific, similar experiments were performed with mouse RAW 264.7 macrophages. As shown previously, these cells also express an osmosensitive betaine transporter, whose activity and BGT-1 mRNA levels are upregulated following hyperosmotic exposure and downregulated during hypoosmolarity [11]. However, in contrast to Kupffer cells, both betaine transport activity and BGT1 mRNA levels are already high during normoosmotic exposure and can only be increased by 20–30% by increasing the osmolarity from 305 to 405 mosmol/l [11]. Like Kupffer cells, RAW 264.7 cells also avidly phagocytose latex particles (Fig. 1B). Comparison between Fig. 1A and B does not suggest major differences in the phagocytotic activity between both cell types. RAW 264.7 mouse macrophages were exposed for 12 h in hyperosmotic medium (405 mosmol/l) and then preloaded with [^{14}C]betaine (10 μmol/l) in the same medium. Following this betaine loading period, cells were exposed to betaine-free medium and the effect of phagocytosis or hypoosmotic exposure on betaine efflux was studied. Over a period of 2 h only 3.1 ± 0.1% ($n = 3$) of the intracellularly accumulated betaine was released in normoosmotic control incubations, whereas this value was 3.5 ± 0.1% ($n = 3$) when simultaneously phagocytosis was stimulated by addition of latex (0.025%, 1 μm diameter). However, when RAW 264.7 cells were exposed to hypoosmotic media (205 mosmol/l), they released 69.8 ± 0.9% ($n = 3$) of the cellular betaine content (Table 3). These data indicate that betaine is released from RAW 264.7 mouse macrophages in response to hypoosmotic cell swelling, but not in response to phagocytosis, as is observed in Kupffer cells.

Similar to the findings in Kupffer cells, addition of latex had no effect on BGT-1 mRNA levels in RAW 264.7 macrophages, whereas hypoosmotic exposure led to a downregulation of BGT-1 mRNA levels (Fig. 3B).

As shown in Table 2, addition of latex to RAW 264.7 mouse macrophages had no effect on cellular betaine uptake. These data indicate that phagocytosis exerts a strong effect on the transport of the osmolyte betaine in Kupffer cells, but not in the RAW 264.7 mouse macrophage cell line.

Table 1
Effect of anisoosmolarity and betaine on phagocytosis of latex particles by cultured rat Kupffer cells over a 1 h period

	Phagocytosis of latex (%)	LDH release (U/l)
205 mosmol/l	121 ± 7[a]	4.6 ± 0.2
305 mosmol/l	100 ± 3	4.7 ± 0.3
405 mosmol/l	64 ± 6[a]	5.1 ± 0.5
405 mosmol/l+0.1 mmol/l betaine	89 ± 6[b]	5.0 ± 0.4
405 mosmol/l+1 mmol/l betaine	104 ± 4[b]	5.0 ± 0.4
405 mosmol/l+10 mmol/l betaine	103 ± 5[b]	4.9 ± 0.3

Kupffer cells were incubated for 13 h in hypoosmotic (205 mosmol/l), normoosmotic (305 mosmol/l) or hyperosmotic (405 mosmol/l) medium, which contained betaine at the concentrations indicated. The osmolarity changes were performed by appropriate changes of the NaCl concentration. During the last 1 h of incubation, fluorescent latex particles (0.025%) were added and phagocytosis was measured as cell-associated fluorescence 1 h after latex addition. Phagocytosis in normoosmotic incubations was set to 100%. Lactate dehydrogenase (LDH) release was measured at the end of the incubations. Data are given as means ± S.E.M. ($n = 4$).
[a]Denotes statistically significant difference ($P < 0.05$) compared to the normoosmotic (305 mosmol/l) control condition.
[b]Significantly different from the hyperosmotic (405 mosmol/l) condition without betaine ($P < 0.05$).

Table 2
Effect of phagocytosis on betaine uptake by Kupffer cells (A) and RAW 264.7 cells (B)

	Betaine uptake (nmol/mg protein per 2 h)	LDH release (U/l)
(A) Kupffer cells		
405 mosmol/l (control)	19.7 ± 0.8	4.7 ± 0.3
405 mosmol/l+14 h latex	7.7 ± 1.2[a]	7.3 ± 1.2
405 mosmol/l+2 h latex	13.3 ± 1.8[a]	5.0 ± 0.4
(B) RAW 264.7 cells		
405 mosmol/l (control)	21.2 ± 0.5	1.6 ± 0.1
405 mosmol/l+14 h latex	20.1 ± 0.8	2.6 ± 0.2
405 mosmol/l+2 h latex	22.5 ± 1.1	2.5 ± 0.2

Kupffer or RAW 264.7 cells were exposed for 14 h to hyperosmotic (405 mosmol/l) medium as described in Section 2 and the uptake of betaine was determined following addition of [^{14}C]betaine (10 μmol/l) during the last 2 h of incubation. As indicated, 0.005% latex particles were present throughout the 14 h hyperosmotic period or 0.025% latex particles only during the last 2 h. Lactate dehydrogenase (LDH) release was measured at the end of the incubations. Data are given as means ± S.E.M. ($n = 3$).
[a]Significantly different from the control ($P < 0.05$).

Table 3
Effect of latex phagocytosis and hypoosmolarity on betaine transport in rat Kupffer cells (A) and RAW 264.7 mouse macrophages (B)

	Betaine efflux (% of total betaine/2 h)	
	(A) Kupffer cells ($n = 6$)	(B) RAW 264.7 cells ($n = 3$)
(a) 405 mosmol/l	27.3 ± 3.5	3.1 ± 0.1
(b) 405 mosmol/l+latex	49.4 ± 3.2[a]	3.5 ± 0.1[a]
(c) 205 mosmol/l	64.8 ± 3.0[a]	69.8 ± 0.9[a]

Cultured Kupffer or RAW 264.7 cells were exposed for 12 h to hyperosmotic (405 mosmol/l) medium as described in Section 2 in order to induce cumulative betaine transport. Then, the cells were preloaded with [^{14}C]betaine (10 μmol/l and 0.5 μCi/ml) for 2 h in hyperosmotic medium. Thereafter, extracellular betaine was removed and the release of cellular betaine (expressed as % of total betaine contained in the cells) was measured following addition of hyperosmotic medium (405 mosmol/l) without (a) or with (b) latex particles (0.025%, 0.1 μm diameter) or following addition of hypoosmotic (205 mosmol/l) medium (c) over a time period of 2 h. Data are given as means ± S.E.M. ($n = 3$ or 6).
[a]Significantly different from the hyperosmotic (405 mosmol/l) condition without latex ($P < 0.05$).

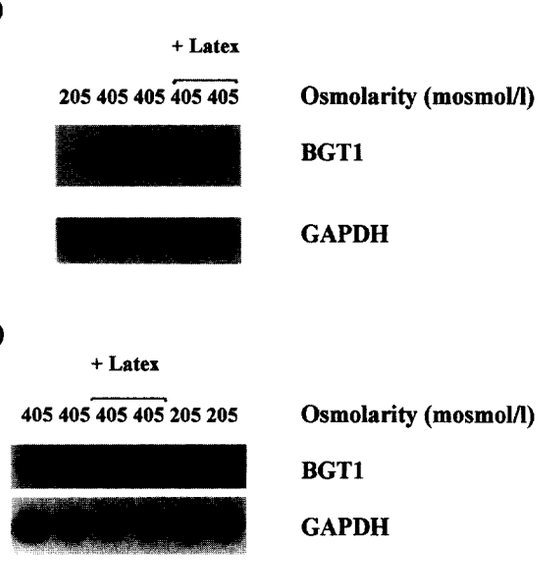

Fig. 3. Effect of latex particles on the hyperosmolarity-induced increase of betaine transporter BGT-1 mRNA levels in rat Kupffer cells (A) and RAW 264.7 mouse macrophages (B). Cultured Kupffer cells (A) or RAW 264.7 cells (B) were exposed for 12 h to hyperosmotic (405 mosmol/l) medium as described in Section 2. 0.0125% latex particles were present throughout the 12 h hyperosmotic exposure of RAW 264.7 cells, or 0.025% latex particles only during the last 2 h of the exposure of Kupffer cells. The osmolarity changes were performed by appropriate changes of the NaCl concentration. Thereafter, the cells were harvested for RNA isolation and subjected to Northern-blot analysis for BGT-1 and GAPDH (7 μg of total RNA per lane).

4. Discussion

This study shows that phagocytosis of latex particles by Kupffer cells is inhibited following hyperosmotic cell shrinkage and that this inhibition can be overcome by betaine, which is known to act as an osmolyte in liver macrophages [10]. The mechanism underlying the osmosensitivity of Kupffer cells' phagocytosis is unclear, as is that underlying the betaine effect. However, betaine was suggested to be a so-called counteracting osmolyte [12–14], i.e. to protect and stabilize protein structures, which could explain the augmentation of phagocytosis by betaine during hyperosmotic stress.

Interestingly, phagocytosis interfered with betaine transport across the cell membrane in Kupffer cells, but not in RAW 264.7 mouse macrophages. In Kupffer cells, phagocytosis stimulated betaine efflux from preloaded cells and diminished betaine accumulation inside the cells, when cells devoid of betaine were exposed to this osmolyte. Phagocytosis, however, did not alter BGT-1 mRNA levels. The most likely explanation is that phagocytosis augments a betaine efflux pathway from the cells, as observed in response to hypoosmotic cell swelling [10,11], but has no effect on unidirectional concentrative betaine uptake via the BGT-1 transporter. Because ingestion of phagocytozable material by Kupffer cells should be accompanied by an increase in cellular volume, these findings could suggest that betaine efflux may be used in these cells for compensation of a phagocytosis-induced cell volume increase. Thus, betaine may not only act as an osmolyte in its classical sense, i.e. a compound which is accumulated or released from the cells in response to anisoosmolarity, but may also contribute to isotonic cell volume homeostasis during phagocytosis in Kupffer cells. Stimulation of betaine efflux during phago-

cytosis was not observed in mouse RAW 264.7 macrophages, indicating that volume-homeostatic betaine fluxes during phagocytosis may not be a feature of macrophages in general. It is unclear whether this difference between 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 only. The latter may well be true when one considers that volume regulation may be by far more critical in sessile Kupffer cells than in other non-sessile macrophages. 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. Accordingly, the interaction between betaine fluxes and phagocytosis in liver macrophages may be a mechanism to maintain liver blood flow. Hypo- and hyperosmolarity of plasma is frequently found in critically ill patients; which may affect the phagocytotic activity liver macrophages. Here, the new perspective arises on a role of osmolytes in modulating immune function.

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