Is fluid-phase endocytosis conserved in hepatocytes of species acclimated and adapted to different temperatures?

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Fluid-phase endocytosis (FPE) serves to recover membrane previously delivered to the plasma membrane via exocytosis. Endocytosis is also central to the immune response (1), uptake of nutrients (31), homeostasis of metabolites (i.e., cholesterol is internalized by receptor-mediated endocytosis (5), synaptic transmission (24), and the modulation of receptor numbers in the plasma membrane (45).

In some cases, the binding of a specific ligand or molecule (such as insulin or epidermal growth factor) to a high-affinity receptor at the plasma membrane triggers internalization. Other receptors (such as receptors for low-density lipoprotein or transferrin) are internalized constitutively, independent of ligand binding. This type of process has been termed receptor-mediated endocytosis (RME) and, in most cases, is mediated via the formation of clathrin-coated vesicles. Alternative pathways for endocytosis also exist that do not involve the formation of clathrin-coated vesicles (22, 27, 42).

The functional significance of FPE is suggested by the remarkable finding that, when clathrin-dependent endocytosis was inhibited in HeLa cells, the activity of alternative endocytic pathways increased in a compensatory fashion to restore similar rates of membrane uptake (9). Accordingly, it has been proposed that FPE plays a constitutive role in maintaining the size of the plasma membrane in most cells by balancing the deposition of membrane (via vesicle trafficking or secretory pathways that target membrane constituents to the plasma membrane) with membrane uptake (26).

Regardless of the internalization pathway, endocytosis is strongly temperature dependent. For instance, in perfused rat liver, the uptake of [125I]asialofetuin was slowed fivefold at 20°C compared with 35°C (10). A similar reduction in endocytic uptake at low temperatures has also been reported for isolated trout hepatocytes, cultured fish macrophages, and mammalian kidney cell lines (29, 40, 41).

The extreme temperature sensitivity of endocytosis poses a significant problem for aquatic poikilotherms, in which body temperature is determined primarily by water temperature (28), because changes in environmental temperature are expected to severely perturb endocytic processes in these organisms. However, the adaptability of aquatic poikilotherms, such as fish, to a wide thermal range and their ability to exhibit normal activity at extremes of temperature suggest that cellular processes, such as endocytosis, may be maintained at appropriate levels following a period of thermal homeostasis.

ENDOCYTOSIS IS A PROCESS CRITICAL to a variety of cellular functions. In secretory cells it serves to recover...
acclimation or adaptation. To test this hypothesis, we studied FPE in hepatocytes isolated from species adapted (trout, Oncorhynchus mykiss, 12°C; tilapia, Oreochromis nilotica, 22–30°C; and the Sprague-Dawley rat, an endotherm) and acclimated (trout, 5 and 20°C; tilapia, 22 and 35°C) to different temperatures. The following questions were addressed. 1) Are rates of FPE comparable (i.e., conserved) when measured at body temperature in species adapted to different temperatures? 2) Is FPE temperature compensated in fish acclimated to different temperatures? We provide evidence that endocytic rates are conserved to some extent in two species of fish (trout and tilapia) both adapted and acclimated to different temperatures. However, when compared at body temperature, rates of endocytosis are significantly higher in a homeotherm (the rat) than the poikilotherms studied.

MATERIALS AND METHODS

Animals

Three different species, rainbow trout (Oncorhynchus mykiss), tilapia (Oreochromis nilotica), and Sprague-Dawley rats were studied in these experiments. Trout were acquired from the Alchesay National Fish Hatchery in White River, AZ, and transported to the laboratory, where they were maintained in circular, fiberglass tanks. Water was recirculated and filtered, and continuous freshwater input resulted in replacement of the total water volume every 24 h. Trout were acclimated for at least 6 wk to a 12-h photoperiod, and water temperatures of either 5 or 20°C. During this period fish were fed ad libitum once daily with commercially available trout food (Supersweet Feed, Minneapolis, MN). Tilapia were purchased from a commercial fish farm (Sweetwater Farms, located on the Gila River Indian Reservation (Maricopa County, AZ), maintained on commercial tilapia food (Integral Fish Foods, Grand Junction, CO), and acclimated to water temperatures of either 22 or 35°C. Sprague-Dawley rats were obtained from the Animal Research Facility at Arizona State University. All procedures for animal use were in accordance with the guiding principles in the care and use of laboratory animals of Arizona State University.

Dietary Treatment

Male Sprague-Dawley rats were obtained from an institutional breeding stock at 35–40 days of age. One group received a nutritionally complete semipurified diet containing 40, 40, and 20% of total kilocalories from fat, carbohydrate, and protein, respectively. The source of the fat was menhaden oil, which is rich in the long-chain polysaturated fatty acid (PUFA) 20:5(n–3) and 22:6(n–3) (n–3 indicates that the first double bond from the methyl end is located at the third carbon). The diet contained 5% of the total fat (wt/wt) as corn oil to prevent essential fatty acid deficiency. The diet was prepared fresh weekly, gassed with nitrogen, and stored at –20°C until use. Animals had free access to food and water, and were housed in wire-bottom cages in a room that was maintained at 25°C on a 12:12-h light-dark cycle. Animals consumed the experimental diet for 4 wk. A control group of rats was maintained under the same conditions and fed with a standard chow diet consisting of 10, 68, and 12% of calories from fat, carbohydrate, and protein, respectively. The source of fat was corn oil.

Isolation of Hepatocytes

Fish. Hepatocytes were prepared by the method of Moon et al. (32). After the animal was anesthetized with a solution of ethyl m-aminoobenzoate [MS-222; 0.5 (trout) or 1.5 g/l (tilapia)] in water, the liver was perfused via the hepatic portal vein in HEPES-buffered (20 mM, pH 7.8) teleost Ringer (concentrations in mM: 176 NaCl, 5.4 KCl, 0.81 MgSO₄, 0.44 KH₂PO₄, 0.35 Na₂HPO₄, 8 NaHCO₃, and 10 glucose) containing heparin (0.2 mg/ml) but lacking calcium until cleared of blood. Perfusion was then continued with a collagenase (Collagenase A, Bohringer Manheim, > 0.15 U/mg) containing (0.4–0.6 mg/ml) teleost Ringer until the liver was well digested (15–20 min). The perfusion media was continuously gassed with a 98% O₂–2% CO₂ mixture. The digested liver was subsequently filtered through nylon mesh of 250 and 74 µm. Cells were collected by centrifugation (100 g for 5 min), washed three times in buffered Ringer, and finally resuspended in HEPES-buffered (20 mM) teleost Ringer supplemented with 2% (wt/vol) BSA.

Rat. Hepatocytes were obtained by collagenase perfusion of the liver as described by Berry and Friend (3) and modified by Blackmore and Exton (4). Briefly, rats were anesthetized with an intramuscular injection of a cocktail containing ketamine, xylazine, and promazine administered at 50, 10, and 5 mg/kg of biomass, respectively. The liver was perfused via the portal vein with calcium-free Krebs-Ringer bicarbonate buffer containing 11 mM glucose and 5 mM pyruvate, which had been equilibrated with 95% O₂ and 5% CO₂ at 37°C (pH 7.4). Once the liver was cleared of blood, 50 ml of the initial perfusate were allowed to drain to waste. Collagenase (0.2 mg/ml, > 0.15 U/mg) was then added to the perfusion system and recirculated until the liver was appropriately digested (10–12 min). The liver was carefully removed and the capsule gently peeled off. The liver was then shaken in 50 ml of Krebs-Ringer bicarbonate buffer containing 1% gelatin to release hepatocytes. Cells were washed three times in the modified Krebs-Ringer buffer with gelatin and resuspended at 3–4 million cells per milliliter. The quality of the cell preparations from fish and rat was assessed by trypan blue exclusion.

Cell Size Measurement

The diameter of hepatocytes from fish and rat was determined by examination under an optical microscope (Olympus BX50, Japan) linked to an image acquisition system (DAGE MTI, Michigan City, IN) and analyzed with the Analytical Imaging Station (AIS) software from Imaging Research (St. Catharines, Ontario, Canada). Cell images were calibrated (in µm) by using an AIS calibration slide.

FPE

The fluorescent dye, 4-amino-N-[3-(vinylsulfonyl) phenyl] naphthalimide-3,6 disulfonyl dibarium salt [Lucifer yellow (LY)] was used as a fluid-phase marker. Cell suspensions (3–4 million cells/ml) were incubated in Erlenmeyer flasks at various temperatures (5 and 20°C for trout, 22 and 35°C for tilapia, and 37, 20, and 10°C for rat) in a medium [fish, HEPES-buffered teleost Ringer, 2% (wt/vol) BSA; rat, Krebs-Ringer buffer, 1% (wt/vol) gelatin] containing LY (0.20–0.25 mg/ml) for 60 min. One-milliliter aliquots were taken at the times indicated in the figure legends, centrifuged at low speed (800–1,000 rpm for 2–5 min), and washed twice in 3 ml of medium lacking LY. This process removed the LY present in the incubation medium but left intact the internalized fraction. The cell pellet was then lysed in 0.5 ml of detergent ( Triton X-100, 0.05% vol/vol). Fluorescence intensity was measured (excitation 430 nm, emission 540 nm, using a
Perkin Elmer LS50B luminescence spectrophotometer), and the rate and extent of internalization and uptake were calculated. A standard curve was used to determine the amount of fluorophore required to give the fluorescent intensities measured in the cell lysates.

Statistical Analysis

All comparisons of endocytic rates were performed by single ANOVA that allows for analysis with different sample size using the SYSTAT software package.

Materials

Collagenase A (from Clostridium histolyticum, activity >0.15 U/mg) was from Boehringer Mannheim (Mannheim, Germany). Gelatin was from DIFCO (Detroit, MI). BSA, LY, glucose, heparin (from porcine intestinal mucosa), sodium pyruvate, trypan blue, HEPES, and MS-222 were from Sigma (St. Louis, MO). Menhaden oil was from ICN Biomedicals. All other chemicals were of analytical grade.

RESULTS

Cell Viability

In both fish species, hepatocytes were at least 98% viable as assessed by trypan blue exclusion. In rat, the cell viability was 85% or greater.

Effect of Acclimation/Adaptation and Assay Temperature on FPE

The kinetics of LY uptake were, in all cases, complex and nonlinear (Figs. 1-3), most likely reflecting the establishment of a steady state between LY internalization by endocytosis and LY elimination via exocytosis. Previous studies with rat hepatocytes (35) demonstrated that endocytosis of LY was nonlinear and that hepatocytes loaded with LY readily secreted the internalized dye. Both the rate and extent of fluid uptake were calculated as indices of endocytosis. Rates were calculated from the first 30 (fish) or 15 (rat) min of uptake, whereas the extent of uptake was defined as the amount of fluid internalized after 60 min of incubation. Rates of endocytosis were measured in freshly isolated hepatocytes after allowing a period of 15 min for stabilization to the assay temperature (AT). In some instances, rates were also measured after cells had been maintained at incubating conditions for 1 h (data not shown). Rates of endocytosis in both cases were the same, indicating that cellular function and endocytic rates were maintained for the duration of our experiments.

Trout

Endocytosis was sensitive to both acute and acclimatory changes in temperature (Fig. 1). The endocytic rate in 20°C-acclimated fish declined from 1.84 pl·cell⁻¹·h⁻¹ at 20°C AT to 1.07 pl·cell⁻¹·h⁻¹ at 5°C AT (see Table 1). However, acclimation to 5°C restored the endocytic rate at 5°C AT to 1.80 pl·cell⁻¹·h⁻¹, corresponding to nearly perfect (95% efficacy) thermal compensation. Rates of endocytosis were equally temperature sensitive in both cold- and warm-acclimated trout, as indicated by Q₁₀ values of 1.40 and 1.43, respectively. The extent of uptake was similarly temperature dependent. In cold-acclimated trout, uptake increased from 0.78 pl/cell at 5°C to 1.57 pl/cell at 20°C. Again following acclimation to 20°C, the extent of uptake was restored to 0.63 pl/cell at 20°C AT, a value similar to that in 5°C trout at 5°C AT.

Tilapia

Rates of endocytosis in hepatocytes of tilapia were more sensitive to acute temperature variation than those in trout hepatocytes (Fig. 2). The endocytic rate
determined at 35°C in 35°C-acclimated tilapia decreased from 3.61 to 1.42 pl·cell⁻¹·h⁻¹ at 22°C (see Table 1). In contrast to trout, acclimation to 22°C resulted in only a partial compensatory adjustment of 36%, as indicated by the endocytic rate of 2.2 pl·cell⁻¹·h⁻¹ determined in hepatocytes of 22°C-acclimated tilapia at 22°C AT. Rates of endocytosis were more sensitive to an acute change in AT in hepatocytes of cold (Q₁₀ of 2.49)- than warm-acclimated tilapia (Q₁₀ of 2.05). The extent of uptake was also temperature dependent and partially temperature compensated. An acute increase in AT from 22 to 35°C increased the extent of uptake from 0.88 to 2.78 pl/cell in 22°C-acclimated tilapia; acclimation to 35°C reduced the level of uptake to 1.38 pl/cell at 35°C AT.

Rat

Both the endocytic rate and the extent of uptake in rat hepatocytes were substantially greater than in fish (Fig. 3). For example, the endocytic rate at 37°C was 16.83 pl·cell⁻¹·h⁻¹, approximately eightfold higher than the rates found in either fish species at their respective body temperatures. Endocytic rates in rat were, however, greatly reduced at low temperature, decreasing to 4.06 and 2.33 pl·cell⁻¹·h⁻¹ at 20 and 10°C, respectively.
corresponding to an average $Q_{10}$ of 2.02. The extent of uptake was similarly temperature sensitive, declining from 4.5 pl/cell at 37°C to 1.66 and 0.60 pl/cell at 20 and 10°C, respectively.

In summary, the temperature sensitivity of rates of FPE based on average $Q_{10}$ values conformed to the rank order: tilapia (2.27) > rat (2.02) > trout (1.41; Table 1). In contrast, the endocytic rates (pl·cell$^{-1}$·h$^{-1}$) determined at the organism's body temperatures were described by the rank order: rat (16.83) > warm-acclimated tilapia (3.61) > cold-acclimated tilapia (2.2) > warm-acclimated trout (1.84) = cold-acclimated trout (1.8). Interestingly, endocytic rates for cold-acclimated tilapia and both warm- and cold-acclimated trout determined at acclimation temperature were relatively similar, whereas endocytic rates at 35°C in 35°C-acclimated tilapia were somewhat (2.5-fold) higher (Table 1); in contrast, endocytic rates in rat at 37°C were considerably greater (approximately eightfold).

**Dietary Effects on FPE in Rats**

The effect of a long-chain PUFA-enriched diet on endocytosis in rat hepatocytes was evaluated by feeding a group of rats with a diet in which menhaden oil represented 40% of the caloric content. Both the endocytic rate and the extent of uptake were higher at 10°C in the long-chain PUFA-fed group as opposed to a chow-fed control group: the endocytic rate increased from 2.33 to 5.35 pl·cell$^{-1}$·h$^{-1}$, whereas the extent of uptake increased from 0.60 to 1.86 pl/cell (Fig. 4).

**DISCUSSION**

We have studied the influence of temperature acclimation and adaptation on rates of FPE in isolated hepatocytes of trout, tilapia, and rat. Rates of endocytosis varied from 1.07 (in cold-acclimated trout) to 16.83 pl·cell$^{-1}$·h$^{-1}$ (in rat). The values reported for rates of endocytosis are highly variable between laboratories, ranging from 0.04 to 30.0 pl·cell$^{-1}$·h$^{-1}$ in the case of rat hepatocytes (14, 33, 35, 36, 43). The reasons for this variability are unclear, but the use of different endocytic markers and methodologies may be contributing factors. Consequently, it is difficult to compare the endocytic rates determined using different markers and/or protocols, and meaningful interspecific comparisons require the use of a standard protocol as employed in the present experiments.

**Effects of Temperature and Thermal Acclimation on FPE**

Endocytosis was sensitive to acute changes in temperature in hepatocytes from both trout and tilapia, although to different degrees. Endocytic rates in hepa-

**Table 1. Rate, extent of uptake, and $Q_{10}$ values for rates of fluid-phase endocytosis in isolated hepatocytes from species adapted and acclimated to different temperatures**

<table>
<thead>
<tr>
<th>Species</th>
<th>Acclimation Temperature, °C</th>
<th>Assay Temperature, °C</th>
<th>$Q_{10}$</th>
<th>Extent of Uptake Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trout</td>
<td>5</td>
<td>5</td>
<td>1.40</td>
<td>0.78 ± 0.02 1.80 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>20</td>
<td>1.43</td>
<td>0.63 ± 0.12 1.84 ± 0.39</td>
</tr>
<tr>
<td>Tilapia</td>
<td>22</td>
<td>35</td>
<td>2.49</td>
<td>0.08 ± 0.12 2.2 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>35</td>
<td>2.05</td>
<td>0.82 ± 0.11 1.42 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>35</td>
<td>1.84</td>
<td>1.38 ± 0.15 3.61 ± 0.18</td>
</tr>
<tr>
<td>Rat</td>
<td>—</td>
<td>10</td>
<td>1.74</td>
<td>0.60 ± 0.25 2.33 ± 0.76</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>20</td>
<td>2.30</td>
<td>1.66 ± 0.14 4.06 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>37</td>
<td>4.50 ± 0.27 16.83 ± 1.4</td>
<td></td>
</tr>
</tbody>
</table>

Rates (pl·cell$^{-1}$·h$^{-1}$) and extent (pl/cell) of uptake are presented as means ± SE for 3–6 experiments performed in duplicate.

Fig. 4. Effect of a long-chain polyunsaturated fatty acid (PUFA)-enriched diet on fluid phase endocytosis in isolated rat hepatocytes. Cells were incubated in medium containing 0.20–0.25 mg LY/ml for indicated times at 10°C. Presented as means ± SE for 3–5 experiments performed in duplicate. Inset: initial rates (pl·cell$^{-1}$·h$^{-1}$) in treatment and control groups; presented as means ± SE of 3–5 experiments performed in duplicate. *Significant difference at $P < 0.05$. 

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ocytes of trout were less affected by a change in temperature (Q_{10} of 1.41) than rates in hepatocytes of tilapia (Q_{10} of 2.27). The temperature sensitivity of endocytic uptake could theoretically reflect the effects of temperature on either endocytic vesicle formation or some downstream process such as lysosomal degradation, which has been shown to be markedly temperature sensitive in trout hepatocytes (41). However, because 1) limitations due to depressed rates of proteolysis at low temperature are more likely to influence rates of RME than FPE, 2) endocytic uptake has been demonstrated at temperatures below the critical temperature for degradation in trout hepatocytes (41), and 3) our estimates of endocytic rates are based on initial rates of LY uptake, we believe these data most likely reflect the inherent temperature sensitivity of vesicle formation and trafficking.

In both fish species, the effects of acute temperature change on endocytic rates were partially offset following a period of thermal acclimation. The extent of the acclimatory adjustment was, however, species specific. Whereas the efficacy of compensation in trout was nearly complete (~95%), tilapia displayed a less-efficient compensatory response (~36%). The smaller effect of AT on rates of endocytosis in trout compared with tilapia may be related to the ability of trout to fully compensate endocytic rates after a period of thermal acclimation. The combined data suggest that trout may be better able than tilapia to deal with the impact of thermal stress on rates of endocytosis.

The machinery responsible for endocytic transport is complex and involves both protein and lipid components. Therefore, mechanisms that account for the observed compensation in endocytic rates could involve either one or, most likely, a combination of both components. Genetic and biochemical approaches have implicated several proteins in the regulation of endocytosis, including coat proteins, rabs (small GTPases), and soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors (SNAREs; for a recent review see Ref. 7). Notably, rab5 has been reported to modulate the kinetics of endocytosis in baby hamster kidney cells (6); cells expressing a mutated rab5 (defective in its GTPase activity) displayed lower rates of FPE, whereas overexpression of wild-type rab5 increased the rate of uptake of endocytic markers. In addition, wortmannin, a potent inhibitor of phosphatidylinositol 3-kinase (PI3K), profoundly inhibits bulk fluid uptake in human fibrosarcoma cells HT-1080 (44).

The protean nature of the machinery that controls vesicle budding and fusion is apparently well conserved between yeast and mammals (7), suggesting that the proteins involved in endocytosis are similar among different taxa of eukaryotes, including fish. Therefore, modulation of the activities of proteins such as rab5 or PI3K in fish, represent potential acclimatory mechanisms responsible for the conservation of rates of endocytic uptake.

Available data in trout provide indirect evidence that membrane lipids may also be involved in the acclimatory adjustment of endocytic rates described in the present study. This evidence includes the compensation of both membrane order (an indication of membrane fluidity) and phase behavior in hepatic plasma membranes of trout acclimated to 5 and 20°C (19, 20) and the remodeling of membrane lipid composition in several trout tissues in response to thermal acclimation. Documented modifications in membrane lipid composition in response to cold acclimation of trout include 1) decrease in the cholesterol-to-phospholipid ratio (39), 2) increase in the degree of fatty acid unsaturation (16), and 3) increase in the ratio of bilayer-destabilizing (BDL) to bilayer-stabilizing (BSL) lipids (18, 21). All of these modifications affect membrane physical properties (by reducing membrane order) and phase behavior (by reducing lipid-phase transition temperatures (8, 13, 38, 46). More importantly, both membrane order and an increased propensity to form nonlamellar phases [i.e., inverted hexagonal (H_{II}) or cubic] have been positively correlated with rates of endocytosis and membrane fusion (critical in endocytic events), respectively (2, 11). Therefore, the acclimatory-induced modifications in membrane lipid composition observed in cold-acclimated trout are consistent with the elevated rates of endocytosis and may actually regulate endocytic rates.

A role for membrane lipids in the regulation of rates of endocytosis is further implicated by manipulation in rats of dietary fatty acid content. Rates of endocytosis in rat hepatocytes were dramatically reduced with decreasing ATs, from 16.83 pl·cell^{-1}·h^{-1} at 37°C to 2.33 pl·cell^{-1}·h^{-1} at 10°C. However, when rats were fed a long-chain PUFA-enriched diet, hepatocytes displayed significantly higher rates of endocytosis (5.35 pl·cell^{-1}·h^{-1} at 10°C AT) than those of rats maintained on the standard chow diet (Fig. 4). Although a direct effect of the dietary treatment on the activity or expression of potential regulatory proteins in hepatocytes (such as rab5 or PI3K) is possible, it has yet to be demonstrated. In contrast, the effects of dietary manipulations on hepatocyte membrane lipid composition are well documented (34, 37). Interestingly, the specific long-chain PUFAs enriched in hepatocyte plasma membranes of PUFA-fed rats [primarily 20:5(n–3) and 22:6 (n–3), unpublished data] are also enriched during cold acclimation in the liver of trout (15), suggesting that specific membrane fatty acids may enhance rates of endocytosis.

To test the hypothesis that membrane fatty acid composition influences rates of endocytosis more definitively, we are currently determining which, if any, lipid modifications occur in purified liver plasma membranes of both trout and tilapia in response to thermal acclimation. If the properties of membrane lipids do regulate endocytosis, one would predict that the degree of lipid remodeling and the compensation of membrane physical properties should be relatively smaller for tilapia than those of trout, reflecting the degree of observed compensation in rates of endocytosis.

Effect of Thermal Adaptation on FPE

Endocytic rates determined at the animal’s body temperature in 5- and 20°C-acclimated trout (1.80 and
1.84 pl·cell$^{-1}$·h$^{-1}$) as well as 22°C-acclimated tilapia (2.22 pl·cell$^{-1}$·h$^{-1}$) were relatively similar (Table 1). Although 35°C-acclimated tilapia assayed at 35°C exhibited a somewhat higher rate (3.61 pl·cell$^{-1}$·h$^{-1}$), these results, especially compared with rat (16.83 pl·cell$^{-1}$·h$^{-1}$), argue for interspecific conservation of endocytic rates in the two species of fish studied. Observations made over the course of the study, such as slower growth rates and lower levels of activity in 35°C-than 22°C-acclimated tilapia suggest that 35°C may be at the upper limits of this species’ temperature tolerance. The median lethal temperature of 38°C reported for Tilapia aurea, another species of tilapia, further supports this interpretation (25). Therefore, it is possible that tilapia at 35°C were in a different metabolic state than those at 22°C, precluding direct comparisons of endocytic rates between the two acclimation groups. This may also explain the partial pattern of thermal compensation for endocytosis (in contrast to the perfect compensation in trout) observed in tilapia.

In marked contrast, endocytic rates in rats, measured at body temperature, were about eightfold higher (16.83 pl·cell$^{-1}$·h$^{-1}$) than in either fish measured at their respective body temperatures. Relatively similar differences have been reported in other physiological processes involving comparisons between endo- and ectotherms. For instance, both standard metabolic rates and rates of oxygen consumption (either total or that attributed to active Na$^+/K^+$ exchange) are four- to sixfold higher in tissue slices of rodents than lizards of the same size (12, 23). Even correcting for differences in cell size (rat hepatocytes were larger (average diameter 21.3 µm) than those of fish (average diameter 12.2 µm)) and expressing endocytic rates per unit of cell membrane surface area (i.e., per micrometer squared), rat hepatocyte membranes still took up fluid (11.81 nl·µm$^{-2}$·h$^{-1}$) approximately three times faster than those of fish (3.85 nl·µm$^{-2}$·h$^{-1}$) at the respective animal’s body temperature. This may indicate that the levels of endocytosis are linked to the organism’s metabolic rate and not necessarily conserved across all taxa.

In contrast, at lower temperatures, endocytic uptake by rat hepatocytes was largely impaired, showing a Q$_{10}$ of 2.02. Interestingly, the Q$_{10}$ in rat was similar to that in tilapia (2.3), with both being considerably greater than the Q$_{10}$ in trout (1.41). This difference may reflect an adaptation of trout to cold conditions. The adaptation of FPE in cold-acclimated fish, in relation to endotherms, is partially evident in the considerably higher endocytic rate found in 5°C-acclimated trout at 5°C AT (3.85 nl·µm$^{-2}$·h$^{-1}$) than that found in rat at 10°C AT (1.62 nl·µm$^{-2}$·h$^{-1}$).

Perspectives

Elucidation of how membrane traffic (MT) is regulated is of basic importance to cell biologists. Investigation of compensatory mechanisms that explain acclimatory responses in endocytic uptake like the ones described in this study may help in understanding how MT is regulated (or altered in pathological states) in other organisms. Several proteins involved in endocytosis have been identified in yeast and mammals (such as rab or phosphatidylinositol kinases), and future study of their potential role in the acclimatory responses reported here may be insightful. In addition, the concepts of homephasic adaptation and dynamic phase behavior predict that membrane physical properties are regulated to maintain proximity of the lamellar phase to both the gel and the H$_{II}$ phase transition temperatures (17, 30); the proximity to the H$_{II}$ phase transition may be of particular importance for endocytic events. This model is consistent with commonly observed patterns of membrane lipid restructuring at low temperature, such as an elevation in acyl chain unsaturation and increase in the BDL/BSL ratio. However, direct evidence of the impact of these types of membrane modifications on endocytosis is still lacking.

Measurement of endocytic rates in hepatocytes whose plasma membrane lipids have been experimentally manipulated may clarify this picture.

Finally, although subject to thermal compensation, it is clear that factors other than body temperature exert a significant influence on rates of endocytosis and perhaps MT in general. The present results suggest that rates of MT may be directly linked to metabolic rate.

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