L-FABP and I-FABP expression increase NBD-stearate uptake and cytoplasmic diffusion in L cells

ERIC J. MURPHY
Department of Physiology and Pharmacology, Texas A & M University, College Station, Texas 77843-4466

Murphy, Eric J. L-FABP and I-FABP expression increase NBD-stearate uptake and cytoplasmic diffusion in L cells. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G244–G249, 1998.—The effects of intestinal and liver fatty acid binding protein (I- and L-FABP, respectively) expression on single-cell fatty acid uptake, internalization, and cytoplasmic diffusion were determined in transfected L cell fibroblasts. These parameters were measured using the nonesterifiable fluorescent fatty acid probe 12-N-methyl-(7-nitrobenz-2-oxa-1,3-diazol)aminostearate (NBD-stearate) and fluorescence digital imaging. In single-cell fluorescence imaging experiments, L-FABP-expressing cells, but not I-FABP-expressing cells, increased NBD-stearate uptake 1.7-fold compared with control cells. Both I- and L-FABP increased the cytoplasmic diffusion rate of the internalized NBD-stearate 2.6- and 1.9-fold, respectively, compared with control cells. However, increased NBD-stearate lateral membrane mobility was observed only in L-FABP-expressing cells. After incubation of the cells with 4 µM NBD-stearate at 37°C for 30 min, fluorescence deconvolution imaging indicated that NBD-stearate was localized primarily into lipid droplets in all cell lines. The differential effect of these proteins on fatty acid uptake and intracellular trafficking in single cells illustrates a possible difference in the physiological function of I- and L-FABP in intact cells.

fatty acid uptake; fatty acid cytoplasmic diffusion; liver fatty acid binding protein; intestinal fatty acid binding protein; fluorescence recovery after photobleaching

The physiological roles of fatty acid binding proteins (FABP) in fatty acid uptake, trafficking, and metabolism have not been fully delineated. Although FABPs have overlapping ligand specificity and sequence homology, the tissue distribution of these proteins is unique (2, 15). One approach to understanding the function of FABP has been to document their ligand binding specificity. FABPs in general bind a wide range of lipophilic molecules, including prostaglandins (5), hydroperoxy- and hydroxyeicosatetraenoic acids (22), heme (25), acyl-CoA (11), and warfarin (17).

For better assessment of the possible physiological functions of FABPs within intact cells, L cells were transfected with cDNA encoding either intestinal (I)-FABP or liver (L)-FABP. With the use of these cells to study fatty acid uptake and esterification, there appears to be a significant functional difference between L- and I-FABP (21). Although I- and L-FABP bind fatty acids with similar affinities, including cis-parinaric acid (4, 18), these two proteins differentially affect cis-parinaric acid uptake in the transfected L cell fibroblasts (21). L-FABP expression significantly increases cis-parinaric and oleic acid uptake into transfected L cells and targets oleic acid esterification to specific phospholipid and neutral lipid classes (16, 21). In contrast, I-FABP expression does not increase cis-parinaric or oleic acid uptake into transfected L cells and targets oleic acid esterification primarily into neutral lipids (20, 21). Increased esterification of oleic acid suggests that both FABPs may facilitate the intracellular movement of fatty acid and/or directly stimulate esterification enzymes in the endoplasmic reticulum.

In the present study, the effects of I-FABP and L-FABP expression on the extent of fatty acid uptake, internalization, and cytoplasmic diffusion were examined using L cell fibroblasts transfected with cDNA encoding either I-FABP (20, 21) or L-FABP (12). With the use of digital fluorescence imaging and 12-N-methyl-(7-nitrobenz-2-oxa-1,3-diazol)aminostearate (NBD-stearate) as a fatty acid probe, the effect of either I-FABP or L-FABP expression on the extent of single-cell fatty acid uptake was determined. Fatty acid localization was assessed using fluorescence deconvolution imaging, and cytoplasmic diffusion was measured using fluorescence recovery after photobleaching (FRAP). The results show that only L-FABP expression increased the extent of fatty acid uptake, but both I- and L-FABP expression dramatically enhanced NBD-stearate cytoplasmic diffusion.

MATERIALS AND METHODS

Cells. Murine L cells (L arpt tk−) were previously transfected with cDNA encoding either I-FABP (20, 21) or L-FABP (12, 16). L-FABP-expressing cells, L-FABP represented 0.35% of the total cytosolic protein (20). L-FABP-expressing cells, L-FABP represented 0.40% of the total cytosolic protein (12). Both control and transfected cells were grown to confluence in Higuchi medium containing 10% fetal bovine serum (Sigma, St. Louis, MO) (8). For digital fluorescence imaging experiments, cells were seeded onto Lab-Tek chambered cover glass slides (Nunc, Naperville, IL) at a density of 25,000 cells/chamber and were used 36 h postseeding. For the steady-state fluorescence stirred cell assay, confluent cells were rinsed twice with PBS, removed by scraping with a rubber policeman, and suspended in PBS at a final concentration of 150,000 cells/ml. For all assays, cell viability was assessed using a combination of phase-contrast microscopy and trypan blue exclusion.

Laser cytometry. Laser cytometry was used to measure the extent of single-cell NBD-stearate uptake. Uptake was measured using an ACAS Ultima (Meridian Instruments, Okemos, MI) equipped with a motorized stage, photomultiplier tube for fluorescence detection, a 1-W argon ion laser (Coherent, Sunnyvale, CA), and an Olympus IM-T inverted epifluorescence microscope (Olympus, Lake Success, NY). For the NBD-stearate uptake studies, the laser power was set at 100 mW, with the excitation beam (488 nm) passing through a 1% neutral density filter. Scan strength was set at 3% (0.03 mW),...
and emission was measured at 530 nm. Before these studies, the instrument was adjusted to generate the maximum fluorescence intensity from the cells while minimizing photobleaching. For all experiments, the stage was maintained at room temperature. The optimal NBD-stearate concentration for measuring maximal uptake in L cell fibroblasts was determined to be 1.0 μM (data not shown). Before each experiment, the cells were removed and washed twice with physiological buffer to remove traces of medium. This buffer contained (in mM) 1.8 CaCl$_2$, 5.0 KCl, 0.9 KH$_2$PO$_4$, 1.0 Na$_2$HPO$_4$, 0.6 MgSO$_4$·7H$_2$O, 6.0 glucose, 138 NaCl, and 10.0 HEPES. Physiological buffer (1 ml) was added to the cells, and the cells were cooled at 4°C for 20 min. Note that cooling the cells before incubation with the probe was done to limit the rapidity of uptake that was seen when the cells were maintained at 37°C. Furthermore, maintaining the cells at 4°C before probe loading did not affect either the cytoplasmic diffusion coefficient or lateral membrane mobility obtained after 15 min on the microscope stage at room temperature, compared with the values for these two parameters obtained for cells maintained at 37°C. Hence, these two parameters were independent of the preincubation temperature, suggesting that the cells maintained at either 4 or 37°C had the same membrane biophysical state at the time probe cytoplasmic diffusion coefficient and membrane mobility were measured. This indicates that the cells were at the same temperature when these parameters were measured and that these parameters were independent of prior storage temperature. After the 20-min incubation, the chamber slides were placed onto the stage and an area containing five or six viable cells was located. After focus was established, the cells were scanned for background fluorescence and NBD-stearate was added to give a final concentration of 1.0 μM. The final ethanol concentration was maintained at ≤0.1% during the experiment. For these experiments, the number of individual cells analyzed, defined as n, was between 32 and 47. This represents an average of five or six individual chamber slides.

Cytoplasmic diffusion. The cytoplasmic diffusion coefficient was determined using FRAP (14). For these studies, NBD-stearate (1 μM) was added as described in Laser cytometry and the cells were incubated at room temperature for 15 min. After incubation, the physiological buffer was removed and the cells were rinsed two times to remove noninternalized NBD-stearate. An area between the nucleus and the edge of the cell was selected for FRAP, and lipid droplets found along the cell periphery were avoided. This area was photobleached with a 15-ms blast by 1 mW of laser power with a beam radius of 1.3 μm. This power effectively bleached 80–90% of the NBD-stearate in that region. Recovery of fluorescence into this region was monitored over time using digital fluorescence imaging, and the diffusion coefficient was calculated using a software algorithm for flat cells provided by the ACAS manufacturer. For these experiments, the number of individual cells analyzed was between 9 and 13, representing 5 or 6 individual chamber slides.

FRAP was also used to measure the lateral mobility within the plane of the membrane, using a lipophilic fluorescent membrane probe (27). For measurement of lateral probe mobility in l-FABP- and L-FABP-expressing cells, a single point on the membrane was photobleached using a 15-ms blast by 1 mW of laser power with a beam radius of 1.3 μm. This power effectively bleached 50–60% of the NBD-stearate in that region. Fluorescence recovery into that region was monitored over time using digital fluorescence imaging. Lateral mobility of NBD-stearate within the plane of the membrane was calculated using a software algorithm for flat cells provided by the ACAS manufacturer. For these experiments, the number of individual cells analyzed was between 17 and 19, representing 5 or 6 individual chamber slides.

Steady-state fluorescence stirred cell assay. The extent of NBD-stearate uptake was also examined using steady-state fluorescence coupled with a stirred cell assay that permits the continuous measurement of cellular NBD-stearate uptake. Harvested cells were kept at 4°C and used for up to 4 h without loss of viability. All measurements were repeated four to six times using the same batch of cells to limit variability due to cell counts. Experimental results were confirmed using a separate set of nonsister cultures. Assays were performed using 300,000 cells per assay suspended in PBS for a final volume of 2 ml. Before the addition of NBD-stearate, the cells were warmed to 37°C for 7 min. Cells were continuously stirred using a Bel-Art cell spin bar (Fisher Scientific, Pittsburgh, PA), with the temperature maintained at 37°C. NBD-stearate was dissolved in ethanol (95% vol/vol) and added directly to the cuvette containing the cells through a septum in the sample compartment lid using a 2-μl syringe (Hamilton, Reno, NV). The final concentration of NBD-stearate was 0.5 μM. This concentration was chosen after incubation of cells with several concentrations of NBD-stearate in a separate set of experiments. The ethanol concentration in the assay did not exceed 0.05%.

Fluorescence intensity data were collected every 0.5 s using a photon-counting fluorimeter (ISS Instruments, Champaign, IL). The excitation wavelength was set at 466 nm, with a 4-nm spectral slit width, and emission was determined through a 530-nm interference filter (Oriel, Stratford, CT).

Intracellular localization. NBD-stearate was localized within the cell using a fluorescence deconvolution imaging workstation (CELLscan; Scanalytics, Billerica, MA) equipped with a Zeiss Axiovert 135TV inverted epifluorescence microscope (Zeiss, Thornwood, NY). Digital images were acquired using wide-field illumination, a charge-coupled device camera, and piezoelectric z-axis control of the objective lens for image collection in a “through focus” series of 45 focal planes at 0.5-μm increments. To reduce photobleaching, the cells were exposed to the light source for 0.1 s and this exposure was regulated by a computer-controlled shutter. Three-dimensional imaging and fluorescence deconvolution algorithms, which use an acquired point-spread function to vector out-of-focus fluorescence to the point of origin in the specimen image, were done using CELLscan software. The point-spread function was measured for the deconvolution algorithm by taking 0.125-μm z-axes intervals of 0.19-μm diameter fluorescent latex beads (Molecular Probes, Eugene, OR).

To differentiate between NBD-stearate localization in mitochondria or lipid droplets, two different probes were used, each of which had specific loading requirements. Nile red (Molecular Probes) was used as a neutral lipid stain that detects neutral lipid droplets (6, 7). Rhodamine 123 was used to visualize mitochondria (3). For Nile red staining, cells in chambered cover glass slides were rinsed two times with physiological buffer and incubated with Nile red (1 μM) at 37°C for 1 min. After this incubation, the cells were rinsed two times to remove excess stain, 1 ml of physiological buffer was added, and fluorescence imaging was done using excitation at 551 nm and emission monitored at 630 nm. Procedures for rhodamine 123 staining were the same as those for Nile red, except the cells were incubated with rhodamine 123 (5 μg/ml) at 37°C for 20 min. Fluorescent images were obtained by probe excitation at 488 nm with emission monitored at 530 nm. For NBD-stearate localization, cells were incubated with NBD-stearate (4 μM) at 37°C for 30 min. Note that these conditions are considerably different from those used in the uptake, cytoplasmic diffusion, and lateral membrane mobility
experiments. All other procedures were done as described. Fluorescence images were obtained by exciting the probe at 488 nm with emission monitored at 530 nm. Because NBD-stearate and Nile red have overlapping excitation and emission wavelengths, colocalization studies using these two probes were not possible.

RESULTS

Laser cytometry. The effects of I- and L-FABP expression on the extent of NBD-stearate uptake were determined using digital fluorescence imaging of single cells. NBD-stearate uptake was significantly greater (P < 0.001) in L-FABP-expressing cells than in I-FABP-expressing and control cells (Table 1). In L-FABP-expressing cells, NBD-stearate uptake was increased 1.7-fold compared with I-FABP-expressing and control cells.

FRAP. The effect of I- and L-FABP expression on cytoplasmic NBD-stearate diffusion was measured using FRAP. This technique can reliably assess the NBD-stearate cytoplasmic diffusion coefficient in living cells (14). In contrast to NBD-stearate uptake, both I- and L-FABP significantly (P < 0.001) enhanced NBD-stearate cytoplasmic diffusion (Table 1). In I-FABP-expressing cells, the NBD-stearate cytoplasmic diffusion rate was increased 2.6-fold compared with control and 1.4-fold compared with L-FABP-expressing cells, respectively. In L-FABP-expressing cells, the NBD-stearate cytoplasmic diffusion rate was increased 1.9-fold compared with control cells. Thus expression of either I- or L-FABP enhanced NBD-stearate cytoplasmic diffusion, although I-FABP appeared to increase the diffusion rate to a greater extent than L-FABP.

FRAP was also used to determine the effects of I- and L-FABP expression on NBD-stearate lateral mobility within the plane of the membrane. Only L-FABP-expressing cells had a significantly (P < 0.05) altered NBD-stearate lateral mobility within the plane of the membrane (Table 2). In L-FABP-expressing cells, lateral mobility of NBD-stearate within the plane of the membrane was increased 1.4-fold compared with control cells. Although I-FABP-expressing cells had a trend for increased lateral mobility, this trend was not statistically significant.

Stirred cell NBD-stearate uptake assay. To confirm the effects of I- and L-FABP expression on fatty acid partitioning into transfected L cell fibroblasts, the extent of NBD-stearate uptake was determined using steady-state fluorescence coupled with a stirred cell assay. NBD-stearate uptake rapidly reached saturation in control, I-FABP-expressing, and L-FABP-expressing cells (Fig. 1). The slight decrease in fluorescence intensity with increasing time represents a combination of probe photobleach and leakage of the probe from the cells. NBD-stearate uptake in L-FABP-expressing cells was significantly (P < 0.001) increased compared with I-FABP-expressing and control cells (Table 3). In L-FABP-expressing cells, NBD-stearate uptake was increased 1.2-fold compared with I-FABP-expressing or control cells. Thus L-FABP, but not I-FABP, expression

### Table 1. Effects of I- and L-FABP expression on NBD-stearate uptake and cytoplasmic diffusion in L cell fibroblasts

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Maximal Fluorescence</th>
<th>Diffusion Rate, 10⁻⁸ cm²/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>782 ± 85</td>
<td>3.38 ± 0.37</td>
</tr>
<tr>
<td>I-FABP expressors</td>
<td>919 ± 63</td>
<td>8.88 ± 0.42†</td>
</tr>
<tr>
<td>L-FABP expressors</td>
<td>1,346 ± 69*</td>
<td>6.31 ± 0.51‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. Maximal fluorescence values were determined using digital fluorescence imaging, and diffusion rates were determined using fluorescence imaging coupled with fluorescence recovery after photobleaching (FRAP). 12-N-methyl-(7-nitrobenz-2-oxa-1,3-diazol)aminostearate (NBD-stearate) concentration was 1.0 µM. *Significantly different from control and intestinal fatty acid binding protein (I-FABP) expressors, P < 0.001, n = 32–47 cells; †significantly different from control and liver (L)-FABP expressors, P < 0.001; ‡significantly different from control, P < 0.001, n = 9–13 cells.

### Table 2. Effects of I- and L-FABP expression on NBD-stearate membrane mobility in L cell fibroblasts

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Membrane Diffusion, 10⁻¹⁰ cm²/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.80 ± 0.36</td>
</tr>
<tr>
<td>I-FABP expressors</td>
<td>4.88 ± 0.42</td>
</tr>
<tr>
<td>L-FABP expressors</td>
<td>5.37 ± 0.50*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 17–19 cells. NBD-stearate concentration was 1 µM, and membrane mobility was determined using digital fluorescence imaging coupled with FRAP. *Significantly different from control, P < 0.05.

---

**Fig. 1.** Representative figure of 12-N-methyl-(7-nitrobenz-2-oxa-1,3-diazol)aminostearate (NBD-stearate) uptake into liver fatty acid binding protein (L-FABP), intestinal (I)-FABP, and control cells, as measured by steady-state fluorescence stirred cell assay. Cell concentration was 300,000 cells/assay, with an assay volume of 2 ml. Cuvette temperature was maintained at 37°C. Excitation and emission parameters were as stated in MATERIALS AND METHODS.
increased NBD-stearate uptake into transfected L cells relative to control cells.

**Intracellular localization.** Intracellular localization of NBD-stearate was determined using fluorescence deconvolution imaging. In all three cell lines, NBD-stearate was primarily found in association with lipid droplets (Fig. 2). To confirm the presence of lipid droplets, cells were incubated with Nile red, a specific lipid droplet stain. Nile red stained round structures located along the periphery of the cell similar to those stained by NBD-stearate (Fig. 3). Rhodamine 123, which was used to visualize mitochondria (Fig. 3), was not colocalized with either Nile red or NBD-stearate (not shown).

**DISCUSSION**

I- and L-FABP expression in L cells differentially affects fatty acid uptake (21) and enhances fatty acid esterification in I- and L-FABP-expressing cells (16, 20, 21), suggesting that I- and L-FABP bind and traffic fatty acids within an intact cell. This possibility is supported by evidence indicating that both I- and L-FABP facilitate fatty acid transfer in vitro between model membranes (10, 24). To better understand the physiological roles of FABP in fatty acid uptake and trafficking, single-cell fluorescence techniques were used to assess these possibilities in L cell fibroblasts expressing either I- or L-FABP.

Although both I- and L-FABP have similar affinities for naturally occurring fatty acids in vitro, these two proteins differentially affect fatty acid uptake in a stirred cell assay (21). The extent of NBD-stearate uptake in single cells was similar to that for cis-parinaric acid or oleic acid uptake (16, 20, 21), which is increased by L-FABP expression but not by I-FABP expression (21). In L-FABP-expressing cells, the extent of NBD-stearate uptake was increased 1.7-fold compared with control cells, as measured by single-cell fluorescence digital imaging. Similar results were obtained with oleic and cis-parinaric acid (16, 20, 21). Hence, fatty acid uptake was increased in L-FABP-expressing cells but not in the I-FABP-expressing cells.

NBD-stearate was also used to determine the effects of FABP expression on the cytoplasmic NBD-stearate diffusion. Cytoplasmic diffusion rates can be measured using several different methods (13, 19), including FRAP (14). Amphipathic molecules such as NBD-stearate have an appreciably slower cytoplasmic diffusion rate compared with diffusion in an aqueous solution (14). This slower rate is attributed to the interaction of the amphipathic molecules with the cytoplasmic matrix, including intracellular membranes (13, 14). This interaction with the cytoplasmic matrix may account for the reported 6.5- to 100-fold slower cytoplasmic diffusion rates compared with rates measured in an aqueous environment devoid of any structural components capable of interacting with the molecule of interest (14, 19, 23).

Unlike fatty acid uptake, NBD-stearate cytoplasmic diffusion was enhanced by the expression of either protein. In I-FABP-expressing cells, NBD stearate diffusion was increased 2.9- and 1.4-fold compared with control and L-FABP-expressing cells, respectively. L-FABP expression increased the NBD-stearate cytoplasmic diffusion rate 1.9-fold compared with control cells. NBD-stearate cytoplasmic diffusion is increased 1.7-fold in female-derived rat hepatocytes compared with male-derived rat hepatocytes (14). This increase was attributed to the higher L-FABP levels found in

---

**Table 3. Effect of I- and L-FABP expression on extent of NBD-stearate uptake in L cell fibroblasts with use of stirred cell assay**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Maximal Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4,833 ± 66</td>
</tr>
<tr>
<td>I-FABP expressors</td>
<td>4,860 ± 86</td>
</tr>
<tr>
<td>L-FABP expressors</td>
<td>5,726 ± 103*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4–6 cells. Maximal fluorescence values were determined using steady-state fluorescence coupled with a stirred cell assay. NBD-stearate concentration was 0.5 µM. *Significantly different from I-FABP expressors and control cells, P < 0.001.
female versus male rat hepatocytes. In I-FABP-expressing Caco-2 cells, there is a twofold increase in the fatty acid transport rate through the cell compared with control cells, suggesting that I-FABP stimulates fatty acid transport (1). The results presented herein showing a 2.6-fold increase in the cytoplasmic diffusion coefficient are consistent with the observed effect in the Caco-2 transfected cells. With the use of a steady-state diaphragm cell cytoplasmic diffusion model, it was found that FABP increased oleic acid diffusion sixfold compared with control cytosol (23). The diffusion coefficients reported were faster than those reported using FRAP, further suggesting that the amphipathic molecule, NBD-stearate, may interact with the cellular matrix, resulting in decreased rates relative to pure cytosol. Thus both I- and L-FABP increased the cytoplasmic diffusion of NBD-stearate, although I-FABP-expressing cells had a significantly greater rate than L-FABP-expressing cells.

The difference in NBD-stearate cytoplasmic diffusion between I-FABP and L-FABP-expressing cells may represent a mechanistic difference between the two proteins. Although both I- and L-FABP facilitate fatty acid transfer between model membranes, I-FABP has a faster transfer rate than L-FABP (10). This faster rate may reflect the difference between two different transfer mechanisms. I-FABP appears to transfer fatty acids by a collisional process in which the protein interacts directly with the membrane, resulting in fatty acid desorption (10). L-FABP appears to transfer fatty acid by an aqueous diffusion process in which the protein binds and transfers the fatty acid after the fatty acid has desorbed from the membrane into the aqueous space (10). Recent structural studies on I-FABP indicate that the protein undergoes significant alterations in structure on fatty acid binding (9). The two proposed structural states of I-FABP may facilitate fatty acid desorption from the membrane and transport of the fatty acid to the proper location. These structural changes are consistent with the results reported by Hsu and Storch (10). Our results suggest that these two proposed mechanisms may be operative within the intact cell.

Because L-FABP expression and I-FABP expression target fatty acid esterification to different lipid classes, continuous expression of these proteins may affect membrane structure. In L-FABP-expressing cells, fatty acid esterification is primarily targeted to phospholipids (16, 21), whereas in I-FABP-expressing cells fatty acid esterification is targeted toward neutral lipids and decreased into phospholipids (20, 21). This increase in fatty acid esterification into phospholipids in L-FABP-expressing cells may account for the changes in plasma membrane lipid composition (26) and the increased fluidity in the plasma membrane fraction purified from L cells expressing L-FABP (12, 26). In the present study, the lateral mobility of NBD-stearate within the plane of the membrane in intact, anchored single cells was increased 1.4-fold in L-FABP-expressing cells compared with control cells. In contrast, I-FABP expression did not significantly increase lateral probe mobility. Because of the apparent limited changes in membrane composition in L cells expressing I-FABP compared with control cells, the lack of a difference in lateral membrane mobility is not surprising. Thus L-FABP expression increased lateral membrane mobility of NBD-stearate within the plane of the membrane in L cells, confirming previous results that showed L-FABP expression increased fluidity of isolated membranes (12, 26).

NBD-stearate intracellular localization was confirmed using fluorescence deconvolution imaging. Nile red preferentially stains lipid droplets (6, 7) and in all cell lines was found localized in round structures along the periphery of the cell. NBD-stearate was localized in structures of similar size, shape, and location as those stained by Nile red, suggesting that NBD-stearate was primarily localized into lipid droplets within the intact cell. Rhodamine, which preferentially stains mitochondria, stained a structurally different organelle and was not colocalized with NBD-stearate, indicating that NBD-stearate was not found in the mitochondria.

Fig. 3. NBD-stearate, Nile red, and rhodamine 123 intracellular localization in I-FABP-expressing cells. Left, NBD-stearate; middle, Nile red; right, rhodamine 123. Note similar location and distribution of Nile red and NBD-stearate staining vesicular structures, identified as lipid droplets. Also note different pattern for NBD-stearate and rhodamine 123 localization, indicating NBD-stearate was absent from mitochondria.
stearate was absent from the nucleus, confirming previous studies (14). Thus NBD-stearate was internalized into the cells and confined to distinct intracellular regions that appeared to be lipid droplets and, to a lesser extent, intracellular membranes.

In summary, these results confirm that the differences in cis-parinaric acid and oleic acid uptake previously observed in L-FABP- and I-FABP-expressing cells reflect differences in uptake rather than in internalization and intracellular diffusion (16, 20, 21). Both proteins increased NBD-stearate cytoplasmic diffusion, whereas only L-FABP expression increased NBD-stearate uptake. These results in intact cells are supported by observations in vitro showing that I- and L-FABP facilitate fatty acid transfer (10, 24). L-FABP-expressing cells had increased NBD-stearate mobility in the membranes compared with control cells, similar to the results reported for plasma membrane fluidity (12, 26). Hence, I- and L-FABP expression enhanced NBD-stearate intracellular cytoplasmic diffusion in an intact cell, suggesting that these proteins are involved in fatty acid trafficking but have differential effects on fatty acid uptake and membrane mobility.

I thank Cindy Murphy for typed preparation of the manuscript. Address for reprint requests: E. J. Murphy, Laboratory of Neuroscience, National Institute on Aging, National Institutes of Health, Bldg. 10, Rm. 6C-103, 9000 Rockville Pike, Bethesda, MD 20892-1582.

Received 17 March 1997; accepted in final form 10 April 1998.

REFERENCES


