δ-Aminolevulinic Acid Transport in Cancer Cells of the Human Extrahepatic Biliary Duct

JANA NEUMANN and MATTHIAS BRANDSCH
Membrane Transport Group, Biozentrum of the Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany

Received November 5, 2002; accepted December 30, 2002

ABSTRACT
This study was performed to characterize the transport of the endogenous photosensitizer δ-aminolevulinic acid in tumor cells of the extrahepatic biliary duct. Uptake of [3H]-δ-aminolevulinic acid into human cholangiocarcinoma SK-ChA-1 cells was linear for up to 10 min, independent of a Na+/H+ gradient, but stimulated 3- to 4-fold by an inwardly directed H+ gradient. Uptake of δ-aminolevulinic acid was mediated by a single transport system with an apparent affinity (K) of 2.1 mM and a maximal velocity (Vmax) of 60.1 nmol·10 min⁻¹·mg of protein⁻¹. Glycylsarcosine, alanylalanine, and cefadroxil strongly inhibited the [3H]-δ-aminolevulinic acid uptake with K values of 1.3, 0.2, and 3.6 mM, respectively. In contrast, γ-aminobutyric acid, glycine, L-glutamic acid, and L-aspartic acid (all 10 mM) had no effect on the total [3H]-δ-aminolevulinic acid uptake, neither at pH 6.0 nor at pH 7.5. Applying a Dixon type of experiment and the ABC test revealed that glycylsarcosine and δ-aminolevulinic acid are transported via the same system, PEPT1. Treatment of the cells with phorbol 12-myristate 13-acetate, a phorbol ester that activates protein kinase C, resulted in a significant inhibition of the transport rate. This inhibition could be blocked by cotreatment with staurosporine. We conclude that δ-aminolevulinic acid is transported by the H+/peptide cotransporter PEPT1 into epithelial cells of the extrahepatic biliary duct. δ-Aminolevulinic acid can be accumulated specifically in bile duct tumor cells before photodynamic therapy.
the kidney, δ-ALA is efficiently reabsorbed after glomerular filtration from the primary filtrate back to the blood. The result was also of high interest for structure-transport considerations of peptide transport because δ-ALA contains a ketomethylene group instead of a peptide bond. Importantly, neither the structurally related GABA, which has a shorter backbone than δ-ALA, nor glutamate could inhibit δ-ALA transport by PEPT1 and PEPT2 (Döring et al., 1998). In rat brain synaptosomes, [3H]δ-ALA, glutamate, and GABA interacted with the same transporter (McLoughlin and Cantrill, 1984). Similarly, for the human adenocarcinoma cell line WiDr transport of δ-ALA by β-amino acid and GABA carriers has been reported (Rud et al., 2000). In amelanotic melanomas uptake of δ-ALA is inhibited by glycine (Langer et al., 1999). Glycine, however, did not inhibit δ-ALA transport in the Döring et al. (1998) study.

Studies regarding the intestinal transport of δ-ALA were extended by Inui’s group. They investigated recognition and transport characteristics of δ-ALA uptake in intact cells (Caco-2) both at the apical and at the basolateral membrane (Irie et al., 2001). They found that cells grown on filters had greater transport activity from the apical-to-basolateral membrane than in the opposite direction. δ-ALA was, however, transported by the basolateral system. The authors did not determine whether GABA, glutamate, glycine, or aspartate affected the total [3H]δ-ALA uptake in Caco-2 cells. At the epithelium of the choroid plexus, it has been described that δ-ALA is transported by two different uptake mechanisms: PEPT2 and a putative Na+/HCO3⁻-dependent organic anion transporter (Novotny et al., 2000).

As stated above, PDT of tumors of the extrahepatic biliary duct is of particular interest. Recently, we described the expression of the intestinal H⁺/peptide symporter PEPT1 in tumor cells of the extrahepatic biliary duct and in normal rabbit bile duct (Knüttler et al., 2002). δ-ALA inhibited uptake of [14C]Gly-Sar via PEPT1. In the present study, we investigated directly the transport characteristics of [3H]δ-ALA in bile duct tumor cells.

Materials and Methods

Materials. The human extrahepatic biliary duct tumor cell line SK-ChA-1 (Knuth et al., 1985) was obtained from the Ludwig Institute for Cancer Research (Zurich, Switzerland). δ-[3,5,3H(N)]amino-levulinic acid ([3H]δ-ALA, specific activity 0.7 Ci/mmol) was purchased from BioTrend (Köln, Germany), [2-3H]glucose (specific activity, 15 Ci/mmol), and [glycine-1-14C]glycylsarcosine ([14C]Gly-Sar, specific radioactivity 53 mCi/mmol) were from Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK). Phorbol 12-myristate 13-acetate (PMA) was supplied by Calbiochem (Germany). Cell culture reagents were obtained from Invitrogen (Germany). All other chemicals were supplied by Sigma Chemie (Deisenhofen, Germany) or Roth (Karlsruhe, Germany).

Cell Culture. Cells at passage number 31 to 59 were maintained in 75-cm² culture flasks at 37°C in a humidified atmosphere with 5% CO₂. They were cultured in minimum essential medium supplemented with nonessential amino acid solution (1%), fetal bovine serum (10%), and gentamicin (50 μg/ml) as described previously (Knüttler et al., 2002). Cells grown to confluence were released by trypsinization (0.05% trypsin/EDTA in modified Pucks solution A) and subcultured in 35-mm disposable Petri dishes (BD Biosciences, Heidelberg, Germany). The medium was replaced every 2 days, the day after trypsinization, and the day before the uptake experiment. With a starting cell density of 0.8 × 10⁶ cells/dish, the cultures reached confluence within 24 h. Uptake was measured 7 days after seeding. Pretreatment of the cells with PMA and/or staurosporine was done in 1.5 ml of medium at 37°C in a humidified atmosphere with 5% CO₂ for 2 h (Brandsch et al., 1994).

Transport Studies. Uptake of [3H]δ-ALA, [14C]Gly-Sar, and [3H]Glycine was determined at 37°C (Knüttler et al., 2002). The uptake buffer was 25 mM 2-(N-morpholino)ethanesulfonic acid/Tris (hydroxymethyl)aminomethane (MES/Tris, pH 6.0) or 25 mM HEPES/Tris (pH 7.5) with 140 mM NaCl or choline chloride, 5.4 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgSO₄, and 5 mM glucose. Uptake experiments were initiated by removing the culture medium from the dishes, washing the cell monolayers twice with 1 ml of buffer, and adding 1 ml of uptake buffer containing [3H]δ-ALA, [14C]Gly-Sar, or [3H]glycine and unlabeled compounds at increasing concentrations. After incubation for the desired time (mostly 10 min), the buffer was removed and monolayers were quickly washed with ice-cold uptake buffer four times, dissolved, and transferred into counting vials. The radioactivity associated with the cells was measured by liquid scintillation spectrometry.

Data Analysis. Each experimental point shows the mean ± S.E. of three to four measurements. The kinetic constants were calculated by nonlinear regression of the Michaelis-Menten plot. The calculated parameters are shown with their S.E. Inhibition constants (Ki) were calculated from IC₅₀ values (i.e., the concentration of the unlabeled compound necessary to inhibit 50% of specific [3H]δ-ALA and [14C]Gly-Sar uptake) using the Kᵢ value of 2.1 mM (obtained in this study) for δ-ALA and 1.1 mM for Gly-Sar (Knüttler et al., 2002). Statistical analysis was done by the two-tailed nonparametric U test. A p < 0.05 was considered significant.

Results

Driving Force of the [3H]δ-ALA Uptake in SK-ChA-1 Cells. Uptake of [3H]δ-ALA (1 μM) into cholangiocytes was strongly stimulated by an inwardly directed pH gradient (Fig. 1). At an outside pH of 6.0, the uptake rate was increased 3- to 4-fold compared with transport at an outside pH of 7.5. The total uptake of [3H]δ-ALA was linear for up to 10 min at pH 6.0.

The uptake of δ-ALA was independent of a sodium gradient. At pH 7.5, the [3H]δ-ALA uptake (3 μM) was 17.7 ± 0.6 pmol·10⁻⁶ mg of protein⁻¹ in the presence of sodium (NaCl buffer). In the absence of sodium (choline chloride buffer), the uptake was 15.3 ± 0.4 pmol·10⁻⁶ mg of protein⁻¹. For the following experiments an incubation time reached confluence within 24 h. Uptake was measured 7 days after seeding. Pretreatment of the cells with PMA and/or staurosporine was done in 1.5 ml of medium at 37°C in a humidified atmosphere with 5% CO₂ for 2 h (Brandsch et al., 1994).

Transport Studies. Uptake of [3H]δ-ALA, [14C]Gly-Sar, and [3H]Glycine was determined at 37°C (Knüttler et al., 2002). The uptake buffer was 25 mM 2-(N-morpholino)ethanesulfonic acid/Tris (hydroxymethyl) aminomethane (MES/Tris, pH 6.0) or 25 mM HEPES/Tris (pH 7.5) with 140 mM NaCl or choline chloride, 5.4 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgSO₄, and 5 mM glucose. Uptake experiments were initiated by removing the culture medium from the dishes, washing the cell monolayers twice with 1 ml of buffer, and adding 1 ml of uptake buffer containing [3H]δ-ALA, [14C]Gly-Sar, or [3H]glycine and unlabeled compounds at increasing concentrations. After incubation for the desired time (mostly 10 min), the buffer was removed and monolayers were quickly washed with ice-cold uptake buffer four times, dissolved, and transferred into counting vials. The radioactivity associated with the cells was measured by liquid scintillation spectrometry.

Data Analysis. Each experimental point shown represents the mean ± S.E. of three to four measurements. The kinetic constants were calculated by nonlinear regression of the Michaelis-Menten plot. The calculated parameters are shown with their S.E. Inhibition constants (Kᵢ) were calculated from IC₅₀ values (i.e., the concentration of the unlabeled compound necessary to inhibit 50% of specific [3H]δ-ALA and [14C]Gly-Sar uptake) using the Kᵢ value of 2.1 mM (obtained in this study) for δ-ALA and 1.1 mM for Gly-Sar (Knüttler et al., 2002). Statistical analysis was done by the two-tailed nonparametric U test. A p < 0.05 was considered significant.

Results

Driving Force of the [3H]δ-ALA Uptake in SK-ChA-1 Cells. Uptake of [3H]δ-ALA (1 μM) into cholangiocytes was strongly stimulated by an inwardly directed pH gradient (Fig. 1). At an outside pH of 6.0, the uptake rate was increased 3- to 4-fold compared with transport at an outside pH of 7.5. The total uptake of [3H]δ-ALA was linear for up to 10 min at pH 6.0.

The uptake of δ-ALA was independent of a sodium gradient. At pH 7.5, the [3H]δ-ALA uptake (3 μM) was 17.7 ± 0.6 pmol·10⁻⁶ mg of protein⁻¹ in the presence of sodium (NaCl buffer). In the absence of sodium (choline chloride buffer), the uptake was 15.3 ± 0.4 pmol·10⁻⁶ mg of protein⁻¹. For the following experiments an incubation time

Fig. 1. pH and time-dependent uptake of [3H]δ-ALA in SK-ChA-1 cells. Uptake of [3H]δ-ALA (1 μM) was measured at pH 7.5 (○) and pH 6.0 (●) for up to 60 min. Values represent means ± S.E. (n = 3–4).
of 10 min and an outside pH of 6.0 (NaCl uptake buffer) was chosen.

**Kinetic Parameters.** The uptake of $[^3H]$δ-ALA was found to be saturable. In the presence of an excess amount of unlabeled δ-ALA (32 mM), uptake was decreased by 85% of total uptake at a 2 μM tracer concentration. This value represents the linear, nonsaturable, nonmediated transport, most likely simple diffusion plus tracer binding. To determine the kinetic parameters of specific δ-ALA uptake, SK-ChA-1 cells were incubated for 10 min with $[^3H]$δ-ALA (2 μM) and increasing concentrations of δ-ALA ranging from 0.3 to 32 mM. The relationship between total uptake rate and substrate concentration is shown in Fig. 2. Kinetic analysis performed by nonlinear regression of total uptake data revealed for the saturable component an apparent affinity constant ($K_a$) of 2.1 ± 0.3 mM and a maximal velocity of transport ($V_{max}$) of 60.1 ± 3.7 nmol · 10 min$^{-1}$ · mg of protein$^{-1}$. The linear, nonsaturable transport constant ($K_0$) was 1.9 ± 0.1 μl · 10 min$^{-1}$ · mg of protein$^{-1}$. Kinetically, there was no evidence for the participation of a second saturable transport component ($p < 0.05$).

**Substrate Specificity of δ-ALA Uptake.** The uptake of $[^3H]$δ-ALA (1 μM) into SK-ChA-1 cells (pH 6.0) could be inhibited by 10 mM unlabeled δ-ALA, Ala-Ala, Gly-Sar, and cefadroxil (Fig. 3). All these compounds are well known substrates of H+/peptide symporters. In contrast, no significant inhibition was found for glycine, GABA, aspartic acid, and glutamic acid at 10 mM concentrations ($p < 0.05$). These compounds, although structurally related to δ-ALA, were not recognized by the system responsible for δ-ALA uptake. Figure 4 shows the results of competition assays performed to determine the apparent affinity constants of the effective inhibitors. From the displacement curves, IC$_{50}$ values were determined by nonlinear regression. From these, the $K_i$ values shown in Table 1 were calculated. The dipeptides and peptidomimetics used displayed apparent affinity constants between 0.2 and 3.6 mM.

**Functional Demonstration of δ-ALA Uptake via PEPT1 in SK-ChA-1 Cells.** To obtain further evidence that δ-ALA is transported exclusively by the intestinal type H$^+$/δ-aminolevulinic acid cotransport in bile duct cells 221 peptide symporter PEPT1 in SK-ChA-1 cells, several types of experiments were performed. In the first series, we determined the type of inhibition of the uptake of the PEPT1

![Fig. 2. Substrate concentration dependence of total δ-ALA uptake in SK-ChA-1 cells. Uptake of $[^3H]$δ-ALA (2 μM) was measured for a 10-min incubation time over a δ-ALA concentration range of 0.316 to 31.6 mM. Inset, kinetic parameters of the saturable transport component. Values represent means ± S.E. (n = 4).](image)

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$[^3H]$δ-ALA $K_i$ values</th>
<th>$[^14]$C Gly-Sar $K_i$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ-ALA</td>
<td>2.7 ± 0.3</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>Gly-Sar</td>
<td>1.3 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Ala-Ala</td>
<td>0.23 ± 0.03</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>Cefadroxil</td>
<td>3.6 ± 0.1</td>
<td>3.4 ± 0.1</td>
</tr>
</tbody>
</table>

Downloaded from jpet.aspetjournals.org on November 19, 2017
substrate Gly-Sar by δ-ALA and its inhibitory constant in a Dixon type of transport study. Uptake of \(^{[14]C}\)Gly-Sar was measured at two different Gly-Sar concentrations (50 and 500 \(\mu M\)) in the presence of increasing amounts of δ-ALA (0–10 \(mM\)). The results are presented as Dixon plot (Fig. 5). They reveal linearity at both Gly-Sar concentrations with lines intersecting above the abscissa in the fourth quadrant, as expected for a competitive inhibitor. A \(K_i\) value of 1.7 \(mM\) was calculated from the point of intersection.

Next, the classical ABC test was performed. For that, in addition to the inhibitory constants of δ-ALA, Gly-Sar, Ala-Ala, and cefadroxil versus \(^{[3]H}\)δ-ALA transport, we determined their \(K_i\) values versus the uptake of \(^{[14]C}\)Gly-Sar (Table 1). These competition assays were performed at an outside pH of 6.0 similar to those of \(^{[3]H}\)glycine (Table 1). Moreover, Ala-Ala and cefadroxil inhibited the uptake of \(^{[3]H}\)δ-ALA and the uptake of \(^{[14]C}\)Gly-Sar with similar potencies, the \(K_i\) values of Ala-Ala being 0.23 \(mM\) (versus δ-ALA) and 0.16 \(mM\) (versus Gly-Sar) and the \(K_i\) values of cefadroxil being 3.6 \(mM\) (versus δ-ALA) and 3.4 \(mM\) (versus Gly-Sar). Hence, all results strictly meet every requirement of the classical ABC test, thus strongly indicating that Gly-Sar and δ-ALA are transported by the same system, PEPT1, in SK-ChA-1 cells.

**Involvement of Other Transport Systems for δ-ALA Uptake in SK-ChA-1 Cells.** As stated above, transport of δ-ALA in SK-ChA-1 cells is sodium-independent. Moreover, there was no significant interaction of glycine, GABA, glutamate, or aspartate with \(^{[3]H}\)δ-ALA uptake at pH 6.0 (Fig. 3). To show unequivocally that PEPT1 is the major or only transport system available for δ-ALA transport in these cells, we also studied the effect of glycine, GABA, glutamate, and aspartate on \(^{[3]H}\)δ-ALA uptake at pH 7.5, i.e., in the absence of a proton gradient. At this pH, uptake of δ-ALA is lower than at pH 6.0. However, just as at pH 6.0, glycine, glutamate, GABA, or aspartate (all 10 \(mM\)) did not affect \(^{[3]H}\)δ-ALA uptake to any significant extent (Table 2). As expected, unlabeled δ-ALA (10 \(mM\)) inhibits \(^{[3]H}\)δ-ALA uptake even in the absence of a pH gradient because under these conditions PEPT1 still transports its substrates to equilibrium. Next, uptake of \(^{[3]H}\)glycine (70 \(nM\)) into SK-ChA-1 cells was measured for 10 min. Unlabeled glycine at a concentration of 10 \(mM\) inhibited \(^{[3]H}\)glycine uptake by 81\% (from 3.5 ± 0.1 to 0.68 ± 0.01 pmol · 10 min\(^{-1}\) · mg of protein\(^{-1}\)). For δ-ALA, we found a weak inhibition of \(^{[3]H}\)glycine uptake by 15\% (to 2.9 ± 0.1 pmol · 10 min\(^{-1}\) · mg of protein\(^{-1}\)) when used at a concentration of 10 \(mM\).

**Effect of Phorbol Ester on δ-ALA Uptake in SK-ChA-1 Cells.** For apical Gly-Sar uptake via PEPT1 in the intestinal cell line Caco-2 it has been described that pretreatment of the cells with phorbol esters inhibits transport of the dipeptide. The inhibition could be blocked by staurosporine (Brandsch et al., 1994). In the present study, we investigated whether uptake of \(^{[3]H}\)δ-ALA is affected by modulators of protein kinase C (Table 3). SK-ChA-1 cells were preincubated with 1 \(\mu M\) PMA or 0.5 \(\mu M\) staurosporine or 1 \(\mu M\) PMA and 0.5 \(\mu M\) staurosporine together, respectively, for 2 h in medium. After washing the cells, \(^{[3]H}\)δ-ALA transport was measured at pH 6.0 using an incubation time of 10 min. Treatment with PMA resulted in a significant inhibition of the \(^{[3]H}\)δ-ALA transport to 73\% (Table 3). Cotreatment with staurosporine completely blocked the PMA effect. Staurosporine alone stimulated the uptake rate significantly by 16\%.

**Discussion**

It has been shown unequivocally by H. Daniels’ group that δ-ALA, although containing a ketomethylene group instead of a peptide bond, represents a substrate for the cloned H+/peptide symporters PEPT1 and PEPT2. Among the various substrates for the intestinal type peptide transporter PEPT1 δ-ALA displays an appreciable affinity of 0.5 \(mM\) when the carrier is expressed in yeast cells and 0.4 \(mM\) when it is

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>(^{[3]H})δ-ALA uptake %</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100 ± 2</td>
</tr>
<tr>
<td>Glycine</td>
<td>91 ± 5</td>
</tr>
<tr>
<td>δ-ALA</td>
<td>71 ± 3*</td>
</tr>
<tr>
<td>GABA</td>
<td>96 ± 3</td>
</tr>
<tr>
<td>Glutamate</td>
<td>112 ± 4</td>
</tr>
<tr>
<td>Aspartate</td>
<td>114 ± 4</td>
</tr>
</tbody>
</table>

\* Significantly different from control.

**Fig. 5.** Determination of the inhibition constant with a Dixon plot. The uptake rate of \(^{[14]C}\)Gly-Sar (10 \(\mu M\)) was measured at pH 6.0 for a 10-min incubation time at two different concentrations of unlabeled Gly-Sar. The diffusional component of 11.6\% measured in the presence of an excess amount of Gly-Sar (50 \(mM\)), was subtracted from the total rate of uptake to calculate the mediated rate of uptake (\(n = 3\)).
A specific system should allow the optimization of treatment of cells, we found no evidence for the participation of transport of \([3H]\)glycine by \(\delta\)-ALA, but compared with the affinity constants of glycine transporting systems, this interaction, in our opinion, is of no physiological significance. Moreover, the kinetic analysis did not suggest presence of more than one transport system.

In conclusion, we obtained evidence that in human cholangiocarcinoma cells SK-ChA-1 \(\delta\)-ALA is transported via the \(H^+\)/peptide symporter PEPT1 into the biliary duct epithelial cells. The mechanism could be a direct phosphorylation/dephosphorylation of the protein or an indirect effect on the \(H^+\) gradient as the driving force of transport (Kennedy et al., 2002).

Acknowledgments

We thank Prof. Dr. A. Knuth (Krankenhaus Nordwest, Frankfurt, Germany) and the Ludwig Institute for Cancer Research (Zurich, Switzerland) for providing the cell line and Prof. Dr. em. Martin Luckner (Halle, Germany) for support.

References


Gonzales P, Tsai PH, and Thibodeau GP (2000) Optimal absorptive systems for glycine, GABA, glutamic acid, or aspartic acid at the total \(\delta\)-ALA transport neither at pH 6.0 nor 7.5. None of these compounds affected the transport of the labeled \([3H]\)\(\delta\)-ALA. We found a small inhibition of the \([3H]\)glycine transport by \(\delta\)-ALA, but compared with the affinity constants of glycine transporting systems, this interaction, in our opinion, is of no physiological significance. Moreover, the kinetic analysis did not suggest presence of more than one transport system.

The inhibitory constants of Ala-Ala, Gly-Sar, cefadroxil, and \(\delta\)-ALA versus \([3H]\)\(\delta\)-ALA and \([14C]\)Gly-Sar transport correspond very well to values published for transport of the dipeptides and pep- tidomimetics via PEPT1 at other cell types (Ganapathy et al., 1995; Irie et al., 2001). The inhibition of Gly-Sar transport by \(\delta\)-ALA was strictly competitive. Furthermore, for the intestinal peptide transporter it has been shown that it is under direct or indirect regulatory control of protein kinase C (Brandsch et al., 1994; Chen et al., 2002). Results presented in this study show that mediators of protein kinase C also affect the \(\delta\)-ALA uptake in bile duct cells. The mechanism could be a direct phosphorylation/dephosphorylation of the protein or an indirect effect on the \(H^+\) gradient as the driving force of transport (Kennedy et al., 2002).

In conclusion, we obtained evidence that in human cholangiocarcinoma cells SK-ChA-1 \(\delta\)-ALA is transported via the \(H^+\)/peptide symporter PEPT1 into the cells. Because PEPT1 is an active transport system, \(\delta\)-aminolevulenic acid might be accumulated in biliary duct epithelial cells against a concentration gradient under physiological or pharmacological conditions before PDT of bile duct cancer.

There are several other systems involved in the transport of \(\delta\)-ALA such as glycine, GABA, aspartic acid, and \(\beta\)-amino acid transporters (McLoughlin and Canterll, 1984; Langer et al., 1999; Novotny et al., 2000; Rud et al., 2000). In SK-ChA-1 cells, we found no evidence for the participation of transport
transport of the dipeptide glycylsarcosine is dependent on functional Na+/H+ exchange activity. Pflugers Arch 445:139–146.


Address correspondence to: Matthias Brandsch, Biozentrum of the Martin-Luther-University Halle-Wittenberg, Membrane Transport Group, Weinbergweg 22, D-06120 Halle, Germany. E-mail: brandsch@biozentrum.uni-halle.de