Agmatine Is Efficiently Transported by Non-Neuronal Monoamine Transporters Extraneuronal Monoamine Transporter (EMT) and Organic Cation Transporter 2 (OCT2)

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ABSTRACT

Agmatine has received considerable attention recently. Available evidence suggests that agmatine functions as a neurotransmitter and inhibits, via induction of antizyme, cellular proliferation. Because of its positive charge, agmatine will not appreciably cross cellular membranes by simple diffusion. Indeed, all physiological models require a channel or transporter protein in the plasma membrane to effect inactivation or non-exocytotic release of agmatine. However, a transport mechanism for agmatine has not been identified on a molecular level so far. In the present study, the non-neuronal monoamine transporters, organic cation transporter (OCT) 1, OCT2, and extraneuronal monoamine transporter (EMT) (gene symbols SLC22A1–A3), both from human and rat, were examined, stably expressed in 293 cells, for [3H]agmatine transport. Our results indicate that OCT2 and EMT, but not OCT1, efficiently translocate agmatine. The structural homolog putrescine was not accepted as substrate. Uptake of agmatine via EMT and OCT2 was saturable, with Km values of 1 to 2 mM. The affinity of OCT1 was 10-fold lower. Carrier-mediated efflux of agmatine results indicate that OCT2 and EMT, but not OCT1, efficiently transport the presence of a channel or transporter protein in the plasma membrane to translocate agmatine. This will allow inactivation of the transmitter by uptake into metabolizing cells, e.g., in the central nervous system, or non-exocytotic release from agmatine-producing cells, e.g., in the kidney. However, so far, a transport mechanism for agmatine has not been identified on a molecular level. We have considered the non-neuronal monoamine transporters EMT, OCT2, and OCT1 (Grünemann et al., 1994, 1996b; Okuda et al., 1996)

In recent years, agmatine has been recognized as an extracellular signaling substance in mammalia. Agmatine [1-(4-aminobutyl)guanidine] is an amine, synthesized by decarboxylation of L-arginine by arginine decarboxylase. Arginine decarboxylase activity is associated with mitochondrial membranes and is most prevalent in kidney, liver, and brain (Lortie et al., 1996). Agmatine is present in plasma and is widely but unevenly distributed in mammalian tissue (Raasch et al., 1995).

There is evidence that agmatine acts as an antiproliferative molecule through induction of the protein antizyme (Satriano et al., 1998; Babal et al., 2001). Antizyme inhibits ornithine decarboxylase and hence polyamine (putrescine, spermine, and spermidine) biosynthesis. At the same time, antizyme suppresses polyamine uptake. Concerted intracellular polyamine depletion eventually leads to growth arrest. Thus, agmatine has been considered a tumor suppressor in the control of cellular proliferation (Satriano et al., 1999). In addition, agmatine might serve as a neurotransmitter or neuromodulator. It is synthesized in specific regions of the brain, stored in synaptic vesicles, released by depolarization, and inactivated by agmatinase and diamine oxidase (Li et al., 1994).

Moreover, agmatine binds to α2-adrenoceptors and imidazoline binding sites, and blocks NMDA receptor channels and other ligand-gated cation channels. It also inhibits nitric oxide synthase and induces release of peptide hormones. Although the precise function of endogenously released agmatine in the central nervous system is presently still unclear, there is obvious therapeutic potential in the treatment of chronic pain (hyperalgesia), addiction, and brain injury (Reis and Regunathan, 2000).

Since agmatine carries one or two positive charges at physiological pH, it will not appreciably cross cellular membranes by simple diffusion. Thus, all physiological models require the presence of a channel or transporter protein in the plasma membrane to translocate agmatine. This will allow inactivation of the transmitter by uptake into metabolizing cells, e.g., in the central nervous system, or non-exocytotic release from agmatine-producing cells, e.g., in the kidney. However, so far, a transport mechanism for agmatine has not been identified on a molecular level. We have considered the non-neuronal monoamine transporters EMT, OCT2, and OCT1 (Grünemann et al., 1994, 1996b; Okuda et al., 1996).

ABBREVIATIONS: EMT, extraneuronal monoamine transporter; OCT, organic cation transporter; MPP+ 1-methyl-4-phenylpyridinium; RT-PCR, reverse transcriptase polymerase chain reaction; h and r, attached to a protein name, designate species as being human or rat, respectively.
as likely candidates. In particular, in a recent study we have demonstrated that OCT2 specifically and efficiently transports guanidine, a main component of agmatine (Gründemann et al., 1999). The aim of the current study, therefore, was to examine whether the non-neuronal monoamine transporters accept agmatine as a substrate. Our results indicate that OCT2 and EMT, but not OCT1, efficiently translocate agmatine across the plasma membrane and may contribute to both release and inactivation of this transmitter.

Materials and Methods

Construction of Expression Vectors for OCT1h and OCT2h. For the cloning of OCT1h, total RNA from a human liver biopsy sample was prepared as described (Gründemann et al., 1997). A cDNA covering the entire open reading frame was generated by RT-PCR with Pwo Polymerase (Roche Diagnostics, Mannheim, Germany) and with primers 5'-GAG AGA GAG TCG CCA CCA TGC CCA CGG TGG ATG ACA TT (forward primer, BamHI site underlined; this primer introduces a perfect XhoI sequence) and 5'-GAG AGA GAG TCG AGC TGC AGA GGA TAA CTC CAT CTT (reverse primer, XhoI site underlined). The amplicon (1.8 kilobases) was cloned, completely sequenced, and eventually assembled into the BamHI and XhoI sites of pcDNA3 (Invitrogen, NV Leek, The Netherlands) to yield pcDNA3OCT1h. The amino acid sequence of our OCT1h clone is identical to the published sequence (GenBank accession number X98332) except for a single amino acid substitution, M408V. Sequencing of three independent clones uniformly revealed the same underlying base disparity, 1294A>G.

Total RNA from human kidney (BD Biosciences Clontech, Palo Alto, CA) was used for the cloning of OCT2h. A cDNA covering the entire open reading frame was generated by RT-PCR with LA Taq mixture (Takara Bio, Shiga, Japan) and with primers 5'-GAG AGA GAG TCG CCA CCA TGC CCA CCA CGG TGG AGC AT (forward primer, BamHI site underlined; this primer also introduces a Kozak sequence) and 5'-GAG AGA GAG TCG AGG GCT CAG GAG TAA GTT TGG TT (reverse primer, XhoI site underlined). The amplicon (1.8 kilobases) was cloned, completely sequenced, and eventually assembled into the BamHI and XhoI sites of pcDNA3 to yield pcDNA3OCT2h. The amino acid sequence of our OCT2h clone is identical to the published sequence (GenBank accession number X98333).

Cell Culture and Transfection. The 293 cells (ATCC CRL-1573), a transformed cell line derived from human embryonic kidney, were grown at 37°C in a humidified atmosphere (5% CO2) in plastic dishes (Nunclon 150288; NUNC A/S, Roskilde, Denmark) precoated with 10% fetal calf serum (Invitrogen). Medium was changed every 2 to 3 days, and the cells were grown at 37°C in 5 mM Tris-HCl, pH 7.4, and radioactivity was determined by liquid scintillation counting, otherwise, in this study all inhibitors were absent during preincubation.

Protein Determination. Protein was measured by a modification of the Bradford method (Zor and Selinger, 1996) with bovine serum albumin as standard.

Calculations and Statistics. Analysis of the time course of substrate accumulation was based on a one-compartment model as described earlier (Russ et al., 1992). A modification for uptake of agmatine into EMTs cells is given in the legend to Fig. 4. Analysis of saturation curves and calculation of K_s values have been reported previously (Schömig et al., 1993).

To model the velocity of uptake as a function of pH (see Fig. 7), for 1-methyl-4-phenylpyridinium iodide (MPP^+), a polynomial of the second degree was used: v = a_0 + a_1 \cdot \text{pH} + a_2 \cdot \text{pH}^2. For agmatine, it follows from S + I = 0.1 \mu M (S = concentration of agmatine with a single positive charge; I = concentration of agmatine with two positive charges), pH = pK_a + log (S/I), and v = v_{max} \cdot S/(K_a + S) (assuming competitive inhibition; with I < K_a and S < K_m, this simplifies to v_I = v_{max}/K_m \cdot S/(1 + \text{pK}_a - \text{pH})). To correct for the pH dependence of the transporter as measured by the uptake of MPP^+ (see above), the right half of the last equation was multiplied with (a_0 + a_1 \cdot \text{pH} + a_2 \cdot \text{pH}^2)/(a_0 + a_1 \cdot 7.5 + a_2 \cdot 7.5^2) = e^{2 \text{pK}_a - 2 \text{pH}}. This effects a normalization relative to pH 7.5, which was chosen arbitrarily. All parameters were fitted by nonlinear regression.

Fitted parameters such as K_m and K_s values are given as geometric mean with 95% confidence interval. Arithmetic means are given with SEM. p values are from a two-tailed unpaired t test.

Drugs. Radiotracers used were agmatine (H-3, 2220 Bq/pmol; ART-608; American Radiolabeled Chemicals, St. Louis, MO), 1-methyl-4-phenylpyridinium iodide (H-3, 2200 Bq/pmol; ART-150; American Radiolabeled Chemicals), and putrescine (H-3, 2960 Bq/pmol; ART-279, American Radiolabeled Chemicals). Unlabeled compounds were agmatine sulfate (10144-3; Aldrich Chemical Co., Steinheim, Germany), MPP^- (0-488; Sigma/RBI, Natick, MA), and putrescine (32810; Fluka, Buchs, Switzerland). Disproportion 24 (1,1-diisopropyl-2,4-cyanine iodide) was synthesized as described previously (Russ et al., 1993). All other chemicals were of analytical grade.

Results

Cloning and Functional Expression of OCT1h and OCT2h in 293 Cells. To comprehensively investigate agmatine transport with the three non-neuronal monoamine transporters OCT1, OCT2, and EMT both from human and rat, we have cloned OCT1h and OCT2h by RT-PCR with specific primers (see Materials and Methods for details) based on the published cDNA sequences (Gorboulev et al., 1997). For functional expression, the cDNAs were inserted into the eukaryotic expression vector pcDNA3. The resulting plasmids were used to stably transfect 293 cells, our standard cell line for heterologous expression of non-neuronal monoamine transporters. Control cells were stably transfected with empty vector. Stably transfected cell lines expressing OCT1r, OCT2r, EMTr, and EMT were available from previous studies (Breidert et al., 1998; Gründemann et al., 1998b, 1999, 2002).

To confirm functional expression of OCT1h and OCT2h, saturation of expressed uptake (i.e., total uptake minus total uptake into control cells) of MPP^+ was examined (Fig. 1). For OCT1h, the apparent Michaelis-Menten constant, K_{m}, was 32 (95% confidence interval, 27–38) \mu M and the maximal uptake was four times with each 4 ml of ice-cold uptake buffer. Subsequently, the cells were solubilized with 0.1% (v/v) Triton X-100 in 5 mM Tris-HCl, pH 7.4, and radioactivity was determined by liquid scintillation counting, otherwise, in this study all inhibitors were absent during preincubation.
Characterization of Agmatine Transport. The above-mentioned cell lines were examined side-by-side in uptake experiments with 0.1 \( \mu \text{M} \) \(^3\text{H}\)Agmatine as substrate. Figure 2, upper row, shows total uptake. To correct for uptake by control cells and for transporter number, the expressed uptake of agmatine was divided by the expressed uptake of \(^3\text{H}\)MPP\(^+\), which was measured in paired assays. The normalized values (Fig. 2, lower row) are directly proportional to transport efficiency (Gründemann et al., 1999). Agmatine is a good substrate, with a factor of about 0.5 relative to MPP\(^+\) uptake, for EMTr and OCT2r, but transport by OCT1r was about 5-fold lower. An analogous pattern (although on a 4-fold lower level relative to MPP\(^+\) uptake) was observed with the human transporters. Here OCT1h was about 9-fold less efficient in the transport of agmatine than EMTh and OCT2h. In contrast to agmatine, uptake of \(^3\text{H}\)Putrescine via EMTr, OCT2r, and OCT1r was not significantly different from control (Fig. 3).

A detailed analysis of the time course of uptake of 0.1 \( \mu \text{M} \) \(^3\text{H}\)Agmatine into stably transfected cells expressing EMTh (Fig. 4) revealed that uptake was linear with time for at least 15 min, but decreased after about 2 h. Uptake into control cells was much lower, with rate constants for inwardly (\( k_{\text{in}} \)) and outwardly (\( k_{\text{out}} \)) directed agmatine fluxes of 1.0 \( \pm \) 0.1 \( \mu \text{mol} \text{ min}^{-1} \text{ mg of protein}^{-1} \) and 0.027 \( \pm \) 0.003 \( \mu \text{mol} \text{ min}^{-1} \text{ mg of protein}^{-1} \), respectively. To take into account the eventual decline in agmatine content, a modified fitting function was used (see Fig. 4 legend). For EMTh-expressing cells, the initial \( k_{\text{in}} \) was 6.7 \( \pm \) 0.3 \( \mu \text{mol} \text{ min}^{-1} \text{ mg of protein}^{-1} \) and \( k_{\text{out}} \) was 0.012 \( \pm \) 0.001 \( \mu \text{mol} \text{ min}^{-1} \). The maximum uptake amounted to approximately 33 \( \mu \text{mol} \text{ mg of protein}^{-1} \). Based on an intracellular water space of 6.7 \( \mu \text{g} \text{ ml}^{-1} \text{ mg of protein}^{-1} \) (Martel et al., 1996) and a conjectured transfection efficiency of 100\%, a 50-fold accumulation of agmatine relative to medium can be estimated. Uptake periods of 1 to 12 min were chosen for subsequent experiments to approximate initial rates of transport.

Expressed uptake of agmatine via OCT2 or EMT both from rat and human was saturable (Fig. 5a, Table 1), with rather uniform values of \( K_m \) (1–2 mM) and \( V_{\text{max}} \) (8–16 nmol min\(^{-1}\) mg of protein\(^{-1}\)). Because of the low transport activity, the affinity for agmatine of OCT1 was determined by inhibition of MPP\(^+\) uptake (Fig. 5b, Table 1). The \( K_m \) values for OCT1r and OCT1h were about 10 times higher than the \( K_m \) values for OCT2 or EMT from the matching species. Thus, compared with OCT1, the affinity of OCT2 and EMT for agmatine is significantly higher.

A trans-stimulation experiment was made to verify that agmatine is actually transported across the plasma membrane. Cells expressing EMTh were preincubated for 20 min in uptake buffer with 1 mM unlabeled agmatine or MPP\(^+\). Control cells were incubated without substrate. After thorough washing, uptake of \(^3\text{H}\)MPP\(^+\) was measured as usual. As expected, EMTh cells preloaded with MPP\(^+\) showed a clear increase in \(^3\text{H}\)MPP\(^+\) uptake versus control (Fig. 6). On the basis of specific uptake, this acceleration of uptake of extracellular radiolabel by counter-transport of unlabeled intracellular substrate amounts to a factor of 1.9 \( \pm \) 0.2 (\( p = 0.0016 \)). With agmatine, stimulation was smaller (factor = 1.4 \( \pm \) 0.2), yet still significant (\( p = 0.029 \)). Thus, it is safe to
conclude that EMT and, by analogy, OCT2 are carriers of agmatine and not mere binding proteins or channels.

Since at physiological pH agmatine molecules with one or two positive charges are present in aqueous solution, we examined which species was the substrate for the non-neuronal monoamine transporters. Initial rates of uptake of 0.1

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**Fig. 3.** Uptake of $[^3H]$putrescine into 293 cells expressing either OCT1r, OCT2r, or EMTr. Control cells had been transfected with pcDNA3, i.e., empty vector. Initial rates of transport were determined for $[^3H]$putrescine and $[^3H]$MPP$^+$ (each at 0.1 μM) in paired assays with an uptake period of 1 min. Shown is the mean ± S.E.M. ($n = 3$).

**Fig. 4.** Time course of agmatine uptake into EMTh cells. Cells grown in dishes were incubated at 37°C with 0.1 μM $[^3H]$agmatine. Shown is the mean ± S.E.M. ($n = 3$). Exponential functions were fitted to the experimental data (Schöning et al., 1992). Open circles, control cells, stably transfected with vector pcDNA3 (see main text for kinetic constants); filled circles, EMTh cells. Model: uptake $= (k_1 - k_2) \cdot \frac{t}{t/\tau + 1} \cdot 0.1 \mu M \cdot (1 - e^{-t/\tau})$; fitting results: $k_1 = 6.7 ± 0.3 \mu l \text{ min}^{-1} \text{ mg of protein}^{-1}; k_2 = 0.012 ± 0.001 \mu l \text{ min}^{-2} \text{ mg of protein}^{-1}; k_{out} = 0.012 ± 0.001 \text{ min}^{-1}$. 

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$[^3H]$putrescine uptake (pmol min$^{-1}$ mg protein$^{-1}$)

$[^3H]$MPP$^+$ uptake (pmol min$^{-1}$ mg protein$^{-1}$)
M[ 3H]agmatine in buffers adjusted to diverse pH values were determined with cells expressing EMT (Fig. 7a). For controls, uptake into cells transfected with empty vector and the pH dependence of MPP\(^{11001}\) uptake was recorded in paired assays. Expressed uptake of the permanent monocation MPP\(^{11001}\) increased slightly with increasing pH, as expected (Scho\¨mig et al., 1992). By contrast, expressed uptake of agmatine was dramatically dependent on pH. The increase in velocity from pH 7.5 to 8.5 amounted to a factor of 7.2\(^{0.5}\). The p\(_{K_a}\) of the amino group of agmatine calculated by non-linear regression was 9.07 (8.84 – 9.44). When agmatine uptake velocity corrected for pH dependence of the carrier protein (as measured with MPP\(^{11001}\) as substrate) was plotted as a function of the concentration of singly charged agmatine (as calculated with the above-mentioned p\(_{K_a}\)) (Fig. 7b), a linear relation was found. In other words, transport velocity was directly proportional to the concentration of agmatine molecules with a single positive charge. This indicates that the agmatine species with two positive charges is no substrate of the non-neuronal monoamine transporters.

**Discussion**

In the present study, uptake of agmatine into control cells as a measure of basal permeability of the plasma membrane was low (expressed as clearance: 0.7 \(\mu\)l min\(^{-1}\) mg of protein\(^{-1}\), cf. Fig. 5). For comparison, the permeabilities of tyramine, MPP\(^{11001}\), histamine, and tetraethylammonium have been recorded in the same system as 3.2 (D. Gr\¨undemann, unpublished observation), 1.1, 0.5, and 0.2 \(\mu\)l min\(^{-1}\) mg of protein\(^{-1}\), respectively (Gr\¨undemann et al., 1999). Thus, there is a definite need for an integral membrane protein to remove agmatine from the extracellular space or to release intracellular agmatine. However, so far, no such transporter has been identified on a molecular level. In the present study we report that the non-neuronal monoamine transporters EMT and OCT2, both from rat and human, efficiently transport agmatine (Fig. 2). By comparison, OCT1 is conspicuously less efficient. The polyamine and spermine precursor putrescine (1,4-diaminobutane) is structurally related to agmatine (1-amino-4-guanidobutane), yet there was no significant transport by any of the (rat) non-neuronal monoamine transporters, which were highly active for MPP\(^{11001}\) transport in paired assays (Fig. 3). We conclude that OCT2 and EMT specifically accept agmatine as substrate, by virtue of its guanidinium residue. This, along with the low activity of

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**Fig. 5.** Affinity of OCT2h and OCT1r for agmatine. a, saturation of expressed uptake of \(^{3}\)Hagmatine\(^{11}\) into 293 cells expressing OCT2h. Uptake into control cells increased linearly with agmatine concentration, slope = 0.68 ± 0.07 \(\mu\)l min\(^{-1}\) mg of protein\(^{-1}\) (\(n = 3\)). See the legend to Fig. 1 for further details. b, inhibition by agmatine of specific MPP\(^{11001}\) uptake into 293 cells expressing OCT1r. Uptake was measured for 1 min to approximate initial rates of transport. Shown as a function of inhibitor concentration is mean ± S.E.M. (\(n = 3\)) of specific uptake of \(^{3}\)H[MPP\(^{11001}\) (0.1 \(\mu\)M) relative to control (no inhibitor present). Specific uptake was defined as that fraction of total uptake that was sensitive to 2 \(\mu\)M disprocynium24.

**TABLE 1**

Affinity for agmatine of the non-neuronal monoamine transporters from rat and human determined with stably transfected 293 cells

<table>
<thead>
<tr>
<th>Transporter</th>
<th>(K_m) or (K_i) 95% C.I.</th>
<th>(V_{max}) mmol/l mg protein(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCT1h</td>
<td>24 ((K_i)) 11, 53</td>
<td>N.D.</td>
</tr>
<tr>
<td>OCT1r</td>
<td>8.6 ((K_i)) 6.1, 12</td>
<td>N.D.</td>
</tr>
<tr>
<td>OCT2h</td>
<td>1.4</td>
<td>0.93, 2.0</td>
</tr>
<tr>
<td>OCT2r</td>
<td>0.95</td>
<td>0.55, 1.6</td>
</tr>
<tr>
<td>EMT(_h)</td>
<td>2.5</td>
<td>1.3, 5.2</td>
</tr>
<tr>
<td>EMT(_r)</td>
<td>0.90</td>
<td>0.58, 1.4</td>
</tr>
</tbody>
</table>

N.D., not determined.
OCT1, in fact utterly resembles experiments with histamine as substrate (Gründemann et al., 1999).

With $K_m$ values of about 1 mM (Table 1), the affinities of EMT and OCT2 for agmatine are comparable with other monoamine transporters such as noradrenaline, histamine, and 5-hydroxytryptamine (Breidert et al., 1998; Gründemann et al., 1998a,b). Since $V_{max}$ values are relatively high, agmatine transport is in a low affinity, high turnover mode. The relatively inefficient transport of agmatine via OCT1 can be fully explained by reduced affinity (measured as $K_i$ values).

Our exemplary trans-stimulation experiment (Fig. 6) provides unambiguous proof that EMTh actually functions as transporter of agmatine, not just as channel or binding protein. Moreover, the experiment clearly documents that agmatine transport also works from inside to outside the cell. We expect similar results for the other non-neuronal monoamine transporters. Interestingly, in a previous study (Gründemann et al., 1999) trans-stimulation of OCT2r by histamine was much stronger (factor = 3.2) than stimulation of EMTh in the present study by agmatine (factor = 1.4), although both compounds gave similar $V_{max}$ values. To clarify this observation, we have examined whether EMTh transports the agmatine species with one or two positive charges. The guanidinium group at one end of agmatine can be considered a permanent cation ($pK_a > 13$) in aqueous solution. We have not found a $pK_a$ for the amino group at the other end of agmatine in the literature, but for putrescine the corresponding $pK_a$ is 8.90 at 37°C. A result from the present study (Fig. 7a) suggests a similar value for agmatine ($pK_a = 9.07$). Thus, protonation of the amino group of agmatine depends very much on pH. For example, the fraction of agmatine molecules with a single positive charge will be 2.6% at pH 7.5 and 21% at pH 8.5. If agmatine$^{2+}$ was the substrate, then uptake velocity should decline with increasing pH. However, the opposite was observed. Agmatine uptake increased dramatically with increasing pH (Fig. 7a) and was directly proportional to agmatine$^+$ concentration (Fig. 7b). Thus, agmatine with a single positive charge is the sole substrate. This fully explains why trans-stimulation was lower with agmatine than with histamine: only a small fraction of intracellular substrate is lower than with histamine. Agmatine$^{2+}$, after all, could be an inhibitor of transport. However, since our data for the determination of $K_m$ and $K_i$ (Fig. 5) do not show any deviation from simple models, we suppose that agmatine$^{2+}$ has no significant affinity for the non-neuronal monoamine transporters. This has further implications. 1) Conversion of the concentration from total agmatine to uncharged agmatine.
The graph shown results from linear regression \( r^2 = 0.9952; n = 5; \) slope = 128 (121–134) \( \mu \text{mol min}^{-1} \text{mg of protein}^{-1} \). The graph shown results from nonlinear regression fitting to models detailed under Materials and Methods.

The time course of agmatine uptake into cells expressing EMTh deserves a mention (Fig. 4). After about 2 h, the intracellular agmatine declines. This is unusual and has not been observed in our laboratory with other substrates. The data could be satisfactorily modeled with the variable

\[ y = \frac{a_0}{1 + \left( \frac{x - a_1}{a_2} \right)^{a_3}} \]

in terms of a cellular mechanism and a more complex definition may be necessary—is the suggestion that uptake capacity decreases over time. We speculate that agmatine triggers changes that eventually affect the carrier protein. Further work is necessary to clarify this phenomenon.

Uptake of \(^{14}\text{C}\)agmatine was studied previously with synaptosomes from whole rat brain, at a pH of 7.2 (Sastre et al., 1997). The transport mechanism was independent of Na\(^+\) and had an apparent \( K_m \) of 19 mM. Whereas 1 mM noradrenaline or dopamine did not inhibit uptake of 0.1 mM agmatine significantly, nonspecific Ca\(^{2+}\) channel blockers Cd\(^{2+}\) or Cd\(^{2+}\) at 1 mM or verapamil at 0.1 mM did so. Noncompetitive inhibition was also achieved by imidazoline receptor antagonists idazoxan and phentolamine (\( K_i = 240 \mu\text{M} \) for the latter). Sastre et al. (1997) concluded that agmatine may be transported through some type of cation channel, especially a Ca\(^{2+}\) channel. However, from our point of view, the agmatine transport mechanism in rat brain may well correspond to either OCT2 or EMT for the following reasons. 1) Affinities of agmatine correlates with transporter expression. 2) Organic cation transport has been shown to be sensitive to heavy metal ions (Katsura et al., 1993). 3) As a large, hydrophobic compound, verapamil likely blocks all non-neuronal monoamine transporters, as demonstrated for EMTh (Martel et al., 2001). 4) With a \( K_i \) of 5 \( \mu\text{M} \), phentolamine is a relatively potent inhibitor, e.g., of EMTh (Gründemann et al., 2002). 5) Both EMT and OCT2 have been detected in brain (Gründemann et al., 1997, 1998b; Busch et al., 1998; Wu et al., 1998; Mooslehner and Allen, 1999). However, the precise localization still has to be established firmly. In the end, additional work is necessary to resolve whether the agmatine transport mechanism from rat brain synaptosomes is in fact EMT or OCT2, and whether distribution of agmatine correlates with transporter expression.

In a recent study with SK-MG-1 cells, an agmatine transport mechanism with a \( K_m \) of 8.6 \( \mu\text{M} \) and a \( V_{\text{max}} \) of 63 nmol min\(^{-1}\) mg of protein\(^{-1}\) was observed (Molderings et al., 2001). Agmatine uptake was independent of Na\(^+\) and could
be inhibited neither by corticosterone nor by O-methyliso- 
pranolol, typical inhibitors of EMT. It was concluded that 
EMT, among others, is not responsible for agmatine uptake 
into SK-MG-1 cells. This is difficult to reconcile with our 
data, however, since EMT is functionally expressed in this 
cell line (Streich et al., 1996). Thus, although the available 
data suggest that in SK-MG-1 cells, a transporter other than 
EMT or OCT2 is responsible for agmatine uptake, some 
additional verification is desirable. In this respect, the 
remarkably steep dependence of agmatine uptake on pH may 
serve as a distinctive functional marker of the non-neuronal 
monoamine transporters. Other recent studies with cultured 
cells suggest the existence of a transporter which, in contrast 
to the non-neuronal monoamine transporters, accepts pu- 
trescine as substrate (Cabella et al., 2001; del Valle et al., 
2001; Satriano et al., 2001). The molecular identity of this 
carrier is presently unknown.

Molecular identification of transporters of agmatine will 
help to explore the versatile actions of this transmitter. For 
example, it has been shown that intracellular agmatine, via 
induction of antizyme, effectively suppresses synthesis and 
uptake of polyamines (Satriano et al., 1998). Since the result-
ing depletion of polyamines inhibits cellular proliferation, 
agmatine may have a role as tumor suppressor. Interest-
ingly, it has been suggested that agmatine could be selec-
tively targeted to rapidly proliferating cells by an increase in 
membrane transport capacity (Satriano et al., 1999). Defin-
ing an agmatine transport system is an important aspect of 
this hypothesis.

In conclusion, we have identified agmatine transport pro-
teins on a molecular level. OCT2 and EMT both from rat and 
human efficiently, specifically, and bidirectionally translo-
cate agmatine across the plasma membrane. By contrast, 
transport of agmatine by the structurally related carrier 
OCT1 is less efficient. From here on, pharmacological target-
ing of OCT2 and EMT may help to elucidate the pleiotrop-
ic functions of agmatine as a signaling substance. Clearly, a 
better understanding of agmatine physiology is indispens-
able for the development of new therapeutic strategies.

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