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ORIGINAL ARTICLE

Agmatine transport in brain mitochondria: a different mechanism from that in liver mitochondria

V. Battaglia · S. Grancara · M. Mancon · C. Cravanzola · S. Colombatto · M. A. Grillo · G. Tempera · E. Agostinelli · A. Toninello

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Abstract The diamine agmatine (AGM), exhibiting two positive charges at physiological pH, is transported into rat brain mitochondria (RBM) by an electrophoretic mechanism, requiring high membrane potential values and exhibiting a marked non-ohmic force–flux relationship. The mechanism of this transport apparently resembles that observed in rat liver mitochondria (RLM), but there are several characteristics that strongly suggest the presence of a different transporter of agmatine in RBM. In this type of mitochondria, the extent of initial binding and total accumulation is higher and lower, respectively, than that in liver; saturation kinetics and the flux–voltage relationship also exhibit different trends, whereas idazoxan and putrescine, ineffective in RLM, act as inhibitors. The characteristics of agmatine uptake in RBM lead to the conclusion that its transporter is a channel with two asymmetric energy barriers, showing some characteristics similar to those of the imidazoline receptor I_2 and the sharing with the polyamine transporter.

Keywords Rat brain mitochondria · Agmatine · Kinetics · Polyamine · Transport · Imidazoline receptor

V. Battaglia · S. Grancara · M. Mancon · A. Toninello (✉)
Department of Biological Chemistry, University of Padova, Viale G. Colombo, 3, 35121 Padova, Italy
e-mail: antonio.toninello@unipd.it

G. Tempera · E. Agostinelli
Department of Biochemical Sciences, Institutes of Biology and Molecular Pathology, University of Rome 'La Sapienza' and CNR, Piazzale Aldo Moro 5, 00185 Rome, Italy

C. Cravanzola · S. Colombatto · M. A. Grillo
Biochemistry Unit, Department of Medicine and Experimental Oncology, University of Turin, Via Michelangelo 27, 10126 Torino, Italy

Abbreviations

ADC	Arginine decarboxylase
AGM	Agmatine
ARG	Arginine
$\Delta\Psi$	Electrical transmembrane potential
$\Delta\mu_{H+}$	Transmembrane electrochemical gradient
DMO	5,5′-dimethyl-oxazolidine-2,4-dione
FCCP	Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone
MAO	Monoamine oxidase
PUT	Putrescine
RBM	Rat brain mitochondria
RLM	Rat liver mitochondria
TPP^+	Tetraphenylphosphonium

Introduction

Agmatine [1-(4-aminobutyl) guanidine] (AGM) is an arginine metabolite, formed by decarboxylation of the amino acid by arginine decarboxylase (ADC) (Reis and Regunathan 2000). Due to its pK_a values, at physiological pH agmatine may be considered as a divalent biogenic amine. The metabolic pathway of its biosynthesis, long known to be present in bacteria, invertebrates and plants, was also recognized in eukaryotes in the mid-1990s (Reis and Regunathan 2000; Raasch et al. 1995). Much evidence has been accumulated regarding the wide range of physiological functions exhibited by agmatine (e.g., Grillo and Colombatto 2004a, b). It is known to bind to α_2-adrenergic and imidazoline receptors and to have properties as a neurotransmitter or neuromodulator (Halaris and Plietz 2007). It stimulates insulin release (Sener et al. 1989) and

induces catecholamines (Sener et al. 1989; Raasch et al. 2001). Agmatine also displays clinical properties, such as neuroprotection and tumor suppression (Satriano et al. 1999); its biochemical functions include induction of ornithine decarboxylase antizyme (Satriano et al. 1998) and spermidine/spermine acetyl transferase (Vargiu et al. 1999), as well as inhibition of nitric oxide synthase (Galea et al. 1996). In mammals, agmatine is not only taken up from exogenous sources through the diet, but is also produced by the intestinal flora (Raasch et al. 1995). Together with its metabolic enzymes, ADC and agmatinase (Regunathan and Reis 2000; Horyn et al. 2005; Sastre et al. 1996), agmatine is present in mitochondria (Gorbatyuk et al. 2001). Indeed, the imidazoline receptor I_2, which binds agmatine, is located on a domain of monoamine oxidase (MAO) of liver mitochondria (Tesson et al. 1995). Agmatine is transported in rat liver mitochondria (RLM) by a specific uniporter displaying electrophoretic behavior and is thought to be a channel or a single-binding centre-gated pore (Salvi et al. 2006). The latter proposal is in line with inhibition studies with charge-deficient agmatine analogs, showing the importance of the guanidine group for agmatine transport (Grillo et al. 2007). Also, previous structural observations of agmatine, obtained with vibrational (Raman) spectroscopy combined with theoretical methods (density functional calculations) (Toninello et al. 2005), strongly support the hypothesis that the transporter is a gated pore. The interactions of agmatine with mitochondria reveal important regulatory effects on the mitochondrial permeability transition (MPT), a phenomenon closely related to apoptosis. In liver, at low concentrations, agmatine amplifies this phenomenon induced by Ca^{2+}, whereas at high concentrations it prevents it (Battaglia et al. 2007). In kidney and brain mitochondria, agmatine exhibits only a protective effect (Arndt et al. 2009; Battaglia et al. 2009). Several important observations about the role of agmatine in brain mitochondria have been obtained, as cited above (Galea et al. 1996; Gorbatyuk et al. 2001; Regunathan and Reis 2000; Horyn et al. 2005; Sastre et al. 1996; Tesson et al. 1995; Salvi et al. 2006; Grillo et al. 2007; Toninello et al. 2005). In this regard, the presence of agmatine and agmatinase in brain mitochondria (Gorbatyuk et al. 2001; Regunathan and Reis 2000; Horyn et al. 2005; Sastre et al. 1996) indicates the activity of a transport system for this amine in RBM, not demonstrated so far. The aim of this study is to demonstrate and characterize this transporter and to assess its kinetic properties versus those identified in RLM (Toninello et al. 2005) which are different from those of other natural polyamines (Toninello et al. 1992). This is in order to verify whether the different effects exerted by agmatine on MPT, previously observed between liver (Salvi et al. 2006) and brain (Battaglia et al. 2009) or kidney (Arndt et al. 2009) mitochondria, may be attributed to different transport mechanisms and membrane interactions, or whether other causes must be invoked.

Materials and methods

Isolation of rat brain mitochondria (RBM)

Rat brain mitochondria were isolated by a conventional differential centrifugation method and purified by the Ficoll gradient method, according to Nicholls (1978), with some modifications. Briefly, rat brain (cerebral cortex) was homogenized in isolation medium (320 mM sucrose, 5 mM HEPES, 0.5 mM EDTA, pH 7.4; 0.3% BSA was added during homogenization and the first step of purification) and subjected to centrifugation (900*g*) for 5 min. The supernatant was then centrifuged at 17,000*g* for 10 min, to precipitate crude mitochondrial pellets. These were resuspended in isolation medium plus 1 mM ATP and layered on top of a discontinuous gradient, composed of 2 ml of isolation medium containing 12% (w/v) Ficoll, 3 ml of isolation medium containing 9% (w/v) Ficoll, and 3 ml of isolation medium containing 6% (w/v) Ficoll. The gradient was centrifuged for 30 min at 75,000*g*. Mitochondrial pellets were suspended in isolation medium and centrifuged for 10 min at 17,000*g*. The pellets were again suspended in isolation medium without EDTA. Protein content was measured by the biuret method with BSA as standard (Gornall et al. 1949). These studies were performed in accordance with the guiding principles in the care and use of animals and were approved by the Italian Ministry of Health.

Standard incubation procedures for RBM

Rat brain mitochondria (1 mg prot/ml) were incubated in a water-jacketed cell at 20°C. The standard medium contained 200 mM sucrose, 10 mM HEPES (pH 7.4), 5 mM succinate, 1 mM phosphate and 1.25 μM rotenone. Variations and/or other additions are described in the legends to the individual experiments presented.

Uptake of agmatine into RBM

Mitochondrial accumulation of [^{14}C]agmatine was determined by a centrifugal filtration method, as previously described (Toninello et al. 1985). The above-mentioned sucrose-based medium was chosen for this study to compare results with those previously obtained in liver (Cabella et al. 2001; Salvi et al. 2006) and also with those for polyamine transport (for a review, see Toninello et al. 2004a, b). The effects of higher ionic strength media were

also determined, with almost similar results. [^{14}C]agmatine was prepared as previously reported (Cabella et al. 2001).

Determination of mitochondrial functions

Electrical transmembrane potential ($\Delta\Psi$) was determined by the distribution of the lipid soluble cation tetraphenylphosphonium (TPP^+) through the inner membrane, measured by a TPP^+ selective electrode prepared in our laboratory, according to previously published procedures (Kamo et al. 1979). ΔpH was measured from the distribution of DMO ([^{14}C] 5,5′-dimethyl-oxazolidine-2,4-dione) through the inner membrane (Rottenberg 1979). The mitochondrial matrix volume was determined from the uptake of [^{14}C]sucrose and ^3H_2O, according to the method of Palmieri and Klingenberg (1979).

Results

Rat brain mitochondria, exhibiting a $\Delta\Psi$ value of about 160 mV (Fig. 1 inset) and a respiratory control index of 4 (result not reported), when incubated in standard medium in the conditions described in "Materials and methods," are able to accumulate agmatine to about 65 nmol/mg prot during 30 min of incubation, after initial binding to the membrane of about 30 nmol/mg prot (Fig. 1). In the presence of de-energizing carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), which collapses $\Delta\Psi$ (Fig. 1 inset), agmatine uptake is almost completely abolished. Strong inhibition of agmatine transport is also observable when the standard medium is deprived of phosphate. This effect is attributable to the drop in $\Delta\Psi$ of about 25 mV, resulting from the absence of phosphate (Fig. 1 inset). In fact, when phosphate is present, its transport into the matrix collapses ΔpH by shifting $\Delta\Psi$ to a higher value (results not reported). These results indicate that agmatine transport in brain mitochondria shows electrophoretic behavior and occurs in conditions of high transmembrane electrochemical gradient ($\Delta\mu_{H+}$) with $\Delta\Psi$ as the driving force. Both phosphate and FCCP reduce the initial binding of agmatine, suggesting possible interference in the binding site of the amine. In general, in studying the transport of polycationic species in mitochondria, a systematic observation is to evaluate if the transporter is specific to or is found in common with other cations. The experimental results of Fig. 2 aim at solving this question in RBM. In this regard, a basic amino acid such as arginine, and a divalent polyamine, such as putrescine, which do not affect $\Delta\Psi$ (Fig. 2 inset), were used as possible inhibitors of agmatine transport. Results show that arginine does not cause any inhibition, suggesting that agmatine and arginine do not share the same transporter. Instead, putrescine induces strong inhibition of the net agmatine transport, about 50%, indicating that the agmatine transporter may be the same as that of putrescine. In order to verify this hypothesis, the effect of agmatine on putrescine transport was also evaluated. The results (Fig. 3) show that agmatine does strongly inhibit putrescine transport, confirming close dependence in the transport of both amines. It should be noted that agmatine does not affect $\Delta\Psi$ (Fig. 3 inset).

For further information on the agmatine transport system in RBM, the influence of other possible effectors such as idazoxan and clorgyline was evaluated. Idazoxan is an inhibitor of the imidazoline receptor I_2 and clorgyline is an inhibitor of MAO. The results (Fig. 4a) show that idazoxan significantly inhibits agmatine transport and favors the initial binding of the amine. Clorgyline strongly inhibits transport and also initial binding. It should be emphasized that putrescine transport is also inhibited by both idazoxan and clorgyline (Fig. 4b), which is consistent with a common transporter for agmatine and putrescine.

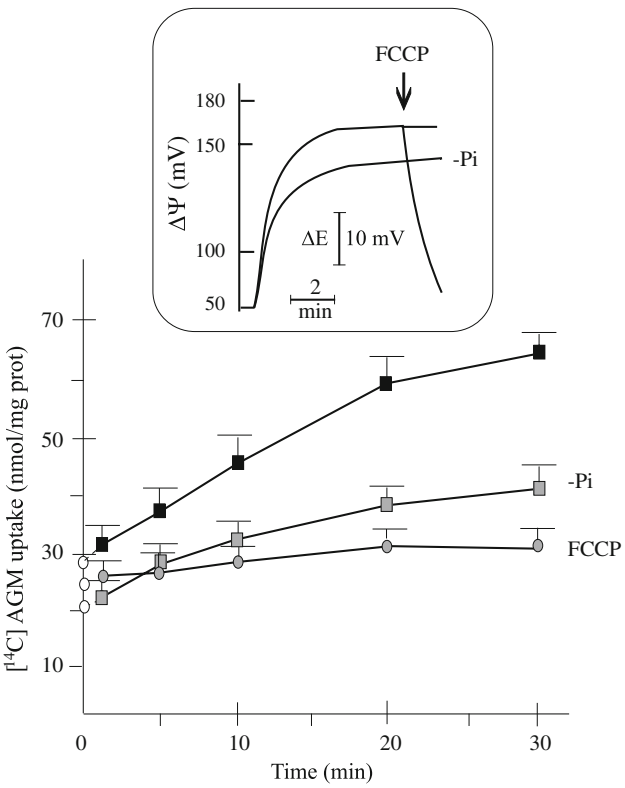

Fig. 1 Agmatine uptake by RBM: dependence on an energized state. Effect of phosphate. Mitochondria were incubated in standard medium, as described in "Materials and methods," with 1 mM [^{14}C]agmatine (50 µCi/mmol) (AGM). When present in medium: 0.1 µg FCCP/mg of protein. *Dotted lines* and *empty circles* on ordinate axis: extrapolation of agmatine binding at zero-time. Values are means ± SD of five experiments. *Inset* determination of mitochondrial membrane potential ($\Delta\Psi$). ΔE = electrode potential

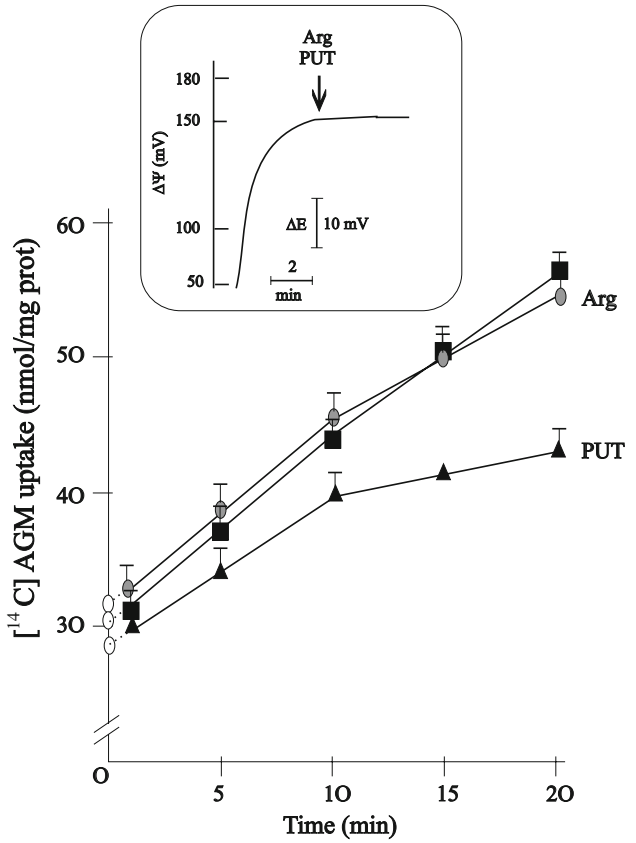

Fig. 2 Effect of polyamine and amino acids on agmatine uptake in RBM. Mitochondria were incubated in standard medium, as described in "Materials and methods," with 1 mM [^{14}C]agmatine (50 μCi/mmol) (AGM). When present in medium: 1 mM putrescine (PUT), 1 mM arginine (Arg). *Empty circles* on ordinate axis: agmatine bound at zero-time. Values are means ± SD of five experiments. *Inset* determination of ΔΨ. ΔE = electrode potential

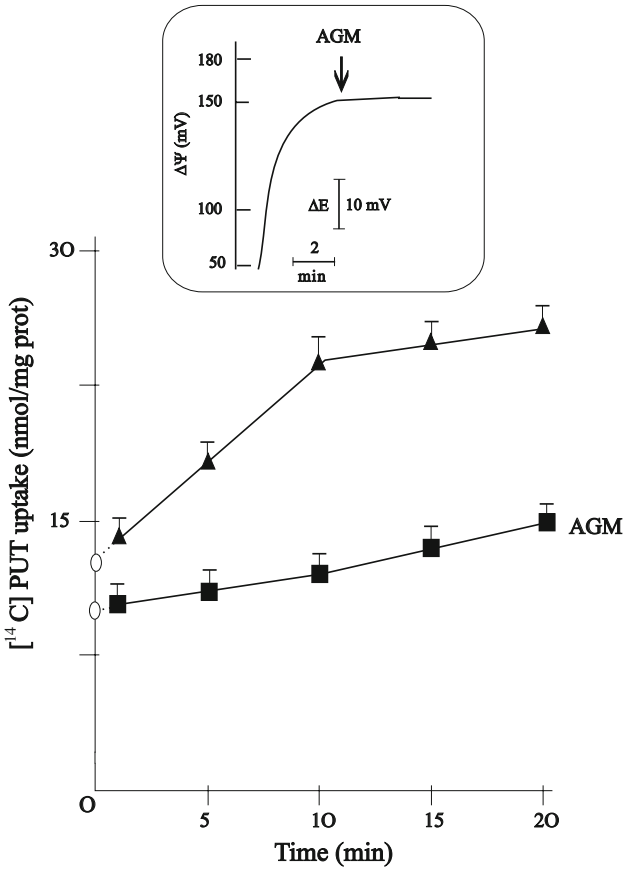

Fig. 3 Putrescine uptake by RBM. RBM were incubated in standard medium, as described in "Materials and methods," with 1 mM [^{14}C]putrescine (50 μCi/mmol). When present in medium: 1 mM agmatine (AGM). *Dotted lines* and *empty circles* on ordinate axis: extrapolation of agmatine binding at zero-time. *Inset* determination of ΔΨ. Values are means ± SD of five experiments

The experiment of Fig. 5a aimed at showing the saturation kinetics of agmatine uptake in RBM, but it fails to show the typical apparent exponential trend observed for agmatine (Salvi et al. 2006) and polyamines (Toninello et al. 1988, 1992, 2004a, b; Agostinelli et al. 2004) in liver mitochondria. In fact, as Fig. 5a shows, agmatine transport versus agmatine concentration exhibits an apparent sigmoidal saturation trend up to the exogenous concentration of 1.5 mM. The calculated $K_{0.5}$ value is 0.6 mM. The presence of both putrescine and idazoxan induces clearcut inhibition of the initial rate of agmatine transport, although without changing the apparent exponential trend and the $K_{0.5}$ value. It is emphasized that, at agmatine concentrations higher than 1.5 mM, the apparent saturation trend fails and transport starts again with a saturation level higher than 2.5 mM (Fig. 5b). In these conditions, putrescine does not exhibit any inhibition—which, conversely, is maintained by idazoxan (compare panels a and b).

The results of Fig. 6a show the dependence of the initial rate of agmatine transport (J) on ΔΨ, which exhibits a much more marked apparent exponential trend than that observed in RLM (Salvi et al. 2006) in the range from 150 to 170 mV. The equation for influx rate, J, in the mitochondrial matrix of a cation is:

$$J = J_0 e^{z\beta F\Delta\Psi/RT} \quad (1)$$

where J_0 is exchange flux (initial transport rate at $\Delta\Psi = 0$), z is agmatine valence, and β is a parameter giving the shape and position of the energy barrier(s) in the membrane for cation transport. For sharp barriers, β equals the fractional distance from the external side of the membrane to the peak of the first barrier (Garlid et al. 1989). The observation of non-ohmic conductance means that flux–voltage analysis can be applied to agmatine transport, as also performed in liver mitochondria (Toninello et al. 1992). Figure 6b shows the semilogarithmic plot of the data of Fig. 6a. This gives an estimate of rate constant k and intrinsic permeability coefficient P, since $J_o = kc$ (J_o is the intercept of the curve on the y-axis and c the concentration of the cation in the medium) and $P = k/400$ (400 is

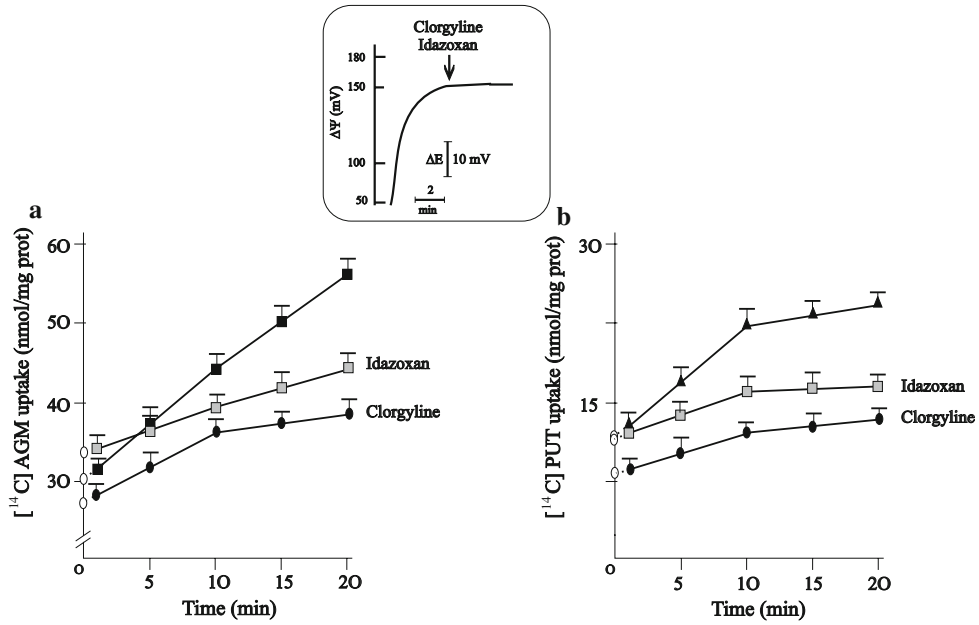

Fig. 4 Effect of idazoxan and clorgyline on agmatine (**a**) or putrescine (**b**) uptake in RBM. RBM were incubated in standard medium, as described in "Materials and methods," with 1 mM [^{14}C]agmatine (50 μCi/mmol) (**a**) or 1 mM [^{14}C]putrescine (50 μCi/mmol) (**b**). When present in medium: 50 μM clorgyline and 200 μM idazoxan. Values are means ± SD of five experiments. *Inset* determination of ΔΨ. ΔE = electrode potential

the active inner membrane surface area measured in cm^2/mg of protein) (Salvi et al. 2006). Assuming that agmatine can cross the membrane with its net charge at pH 7.4, that is 1.98 (the pK_a values of agmatine are 9.07 and >13) (Grundemann et al. 2003) but also as a monovalent cation, the values of β can be estimated from the relative slope $z\beta$ of the curve shown in Fig. 6b. All the above parameters are listed in the table inserted in the figure.

It should be emphasized that the agmatine concentrations used in these experiments are probably far higher than those physiologically present in neurons. However, as the density of isolated mitochondria suspended in the standard medium is also far higher than that normally present in the cytosol, high agmatine concentrations were used in this type of experiments. Indeed, as the present study was performed in order to compare agmatine transport in RBM with that in RLM, it was necessary to use the same concentrations of agmatine as those used in studies with RLM (Salvi et al. 2006).

Discussion

The results reported here provide evidence that agmatine is gradually taken up into the matrix space of energized RBM (Fig. 1) revealing differences between agmatine transport in RBM and RLM; RLM exhibits higher accumulation, but RBM exhibits higher initial binding [compare results in RLM (Salvi et al. 2006)]. Indeed, the presence of an electrogenic mechanism is shown, closely depending on the initial rate of agmatine transport on ΔΨ (Figs. 1, 6) and resembling that found in RLM (Salvi et al. 2006). In consideration of the characteristics of agmatine transport in RLM, one of the most important is that this process is mediated by a specific transporter for this amine (Salvi et al. 2006). Instead, the present results show that, in RBM, the agmatine transporter is shared with the divalent putrescine (Figs. 2, 3), while the amino acid arginine, as in RLM, has another transporter (Fig. 2). Again, in comparison with agmatine transport in RLM, the results obtained with idazoxan and clorgyline reveal other important and different characteristics of agmatine uptake in RBM. As previously reported, idazoxan is ineffective in inhibiting agmatine transport in RLM (Salvi et al. 2006). Instead, in RBM, idazoxan markedly inhibits agmatine accumulation (but not binding) (Fig. 4a), without changing mitochondrial ΔΨ, thus excluding any effect linked to the energy of the transport. This result, when considered with the inhibition caused by the presence of clorgyline (Fig. 4a) and taking into account the fact that the I_2 receptor is located on a MAO domain (Tesson et al. 1995), supports the hypothesis that the agmatine transporter in RBM may be identified with a system having some characteristics of the above-mentioned receptor. This proposal is further supported by the observation that putrescine transport, which is shared with that of agmatine, is also inhibited by both idazoxan and clorgyline (Fig. 4b).

Kinetic analyses of agmatine uptake in RBM indicate that the transporter involved in this mechanism may have the structure of an oligomeric protein, exhibiting positive coordination for its function. Thus, very probably, this transporter is composed of more than one subunit, and more than one transport site. The approximate sigmoidal trend of the dose-dependent initial rate of agmatine transport (Fig. 5a) indicates an apparent cooperative effect, with idazoxan and putrescine as negative effectors at low

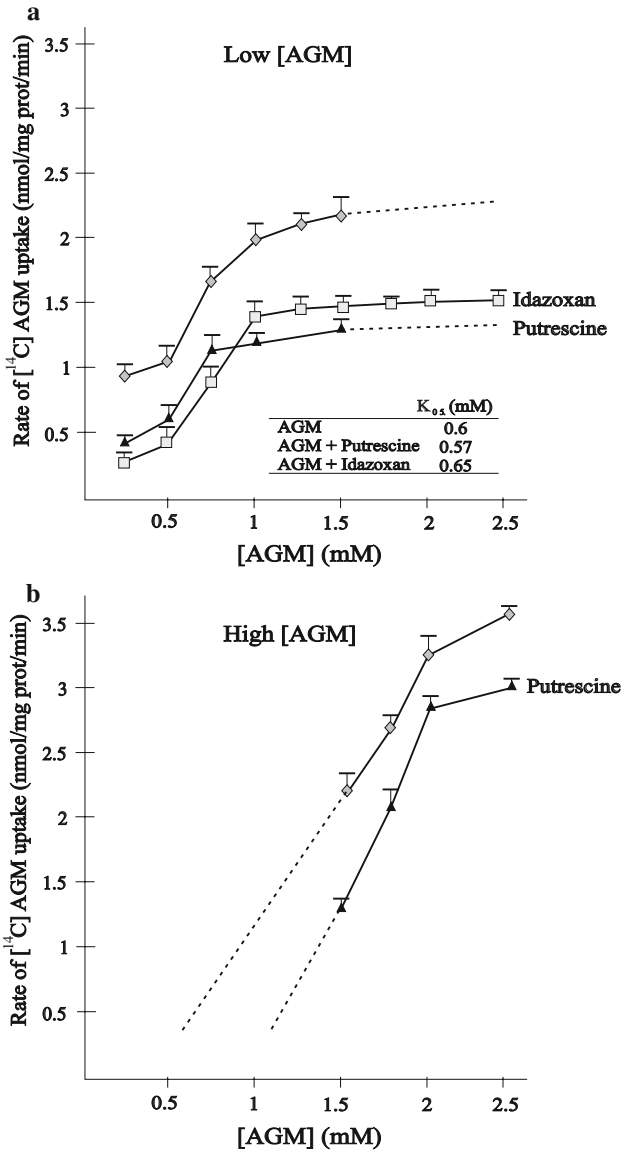

Fig. 5 Saturation kinetic of agmatine uptake in RBM. RBM were incubated for 5 min in standard medium, as described in "Materials and methods," with [^{14}C]agmatine (50 μCi/mmol) at indicated concentrations. **a** data for agmatine concentrations <1.5 mM, **b** >1.5 mM. When present in medium: 1 mM putrescine and 200 μM idazoxan. Agmatine uptake at all concentrations was linear over incubation period. Values are means ± SD of three experiments

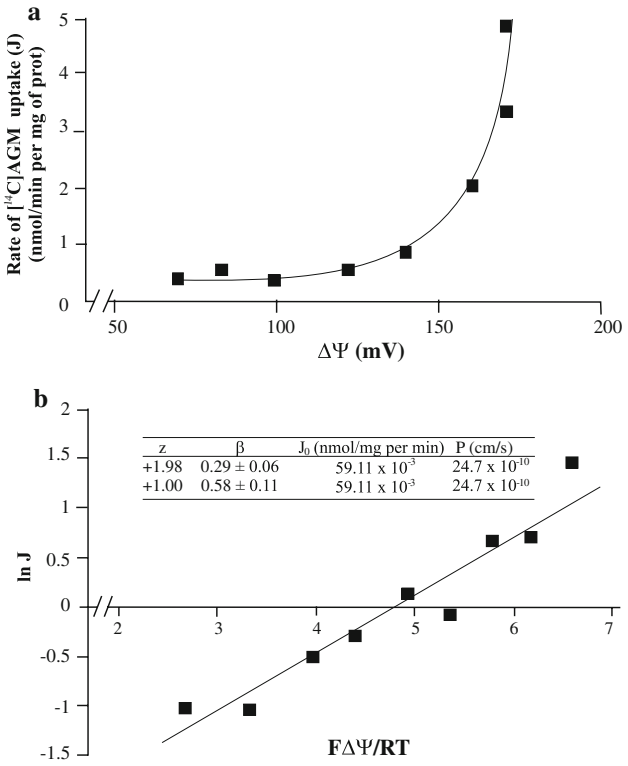

Fig. 6 Flux–voltage relationship and flux–voltage analysis for agmatine uptake. **a** Agmatine flux (nmol/min per mg prot) (J) plotted versus ΔΨ. RBM were incubated in standard medium with 1 mM [^{14}C]agmatine (50 μCi/mmol). ΔΨ was modulated by adding limiting amounts (10–60 nM) of FCCP to decrease it or by adding 0.33 μg/mg prot nigericin to reach highest value. Amine uptake values were corrected for instantaneous electrostatic binding (Toninello et al. 1988). Agmatine uptake was linear over incubation period. **b** Log-linear plot of data in panel **a**. Linear regression analysis gave values for slope of curve representing product $z\beta$ (see Eq. 1). Intercepts of curve on ordinate axis gave values of ln J_0. A representative experiment is shown; six other experiments gave almost identical results

agmatine concentrations. It should be noted that, at concentrations higher than 1.5 mM, a further increase in the rate of agmatine transport is observed (Fig. 5b), suggesting the presence of more than one autoregulatory site for agmatine in its transporter. In the presence of idazoxan, inhibition of agmatine transport is also maintained at high concentrations, further confirming the above hypothesis about the nature of the transporter (Fig. 5a). Further amplification of agmatine transport by putrescine, observed at high agmatine concentrations, may be explained by the positive cooperation induced by putrescine on the agmatine transporter.

The flux–voltage relationship of agmatine transport (Fig. 6a) highlights another significant difference between RBM and RLM. In RBM, the ΔΨ threshold is between 150 and 170 mV, which accounts for most of the agmatine uptake. Instead, in RLM, the exponential trend is much lower and the ΔΨ range involved in the transport is from 140 to 210 mV. This marked difference between the two types of mitochondria clearly demonstrates the different nature of the structure of the agmatine transporters in RBM and RLM. Considering flux–voltage analyses (Fig. 6) it should be noted that agmatine, depending on the valence of the transported species, can exhibit two different β values (Fig. 6b inset). The calculation of $\beta = 0.29$ for the divalent form, very close to that of the transport in RLM ($\beta = 0.25$) (Salvi et al. 2006), suggests that agmatine is transported by

a channel with two energy barriers, similar to that of natural polyamines (Toninello et al. 1992). This possibility is also supported by the calculation of exchange flux J_0 and intrinsic permeability coefficient P (Fig. 6b inset). These values—59.11 × 10^{-3} nmol/mg min and 24.63 × 10^{-10} cm/s, respectively—are very similar to those calculated in RLM (Salvi et al. 2006). Thus, taking into account the previous reasoning regarding polyvalent cation transport (Toninello et al. 1992; Salvi et al. 2006), we propose the presence of a uniport transporter, which may be a channel. However, force–flux analyses also demonstrate that monovalent agmatine may be taken up by a transport system having a β value of 0.58 (Fig. 6b inset), very similar to that calculated in RLM, which is 0.5 (Salvi et al. 2006). This β value, as also considered in RLM, may be applied to a single-binding centre-gated pore of which a well-known example is the ATP/ADP translocator (Klingenberg 2005). That this type of transporter may be identified with that of agmatine is supported by the fact that monovalent agmatine has a very high dipole moment ($\mu = 15.8$) when compared with the divalent form ($\mu = 5.1$ D) (Toninello et al. 2005), whereas polyamines have $\mu = 0$. These parameters indicate that the monovalent form is more probable for an electrophoretic mechanism. The latter hypothesis, subsequently supported by experiments with agmatine analogs (Grillo et al. 2007), was taken into account in proposing the type of transporter in RLM (Salvi et al. 2006; Grillo et al. 2007). However, all the observed differences in the transport between RBM and RLM—that is, the extent of initial binding and total accumulation, kinetics, force–flux relationships, and the effects of inhibitors—strongly support the substantial difference in the nature of the transporter. Thus, considering all the above, the agmatine transporter in RBM should also have different β parameters, so that it may be a transport system identifiable with a channel having two asymmetric energy barriers, as demonstrated by the flux–voltage analyses which consider a β value of 0.29 (see above).

In conclusion, these results open new possibilities regarding the mechanism of agmatine transport, and suggest the presence of a transporter in RBM significantly different from that of RLM and having some characteristics of the I_2 receptor. The hypothesis for the involvement of this receptor in the transport cannot yet be proposed, as it is not demonstrated by the results reported here. Certainly, this possibility would answer the question: on the role of I_2 receptor in mitochondria and its binding with agmatine. Further experiments are in progress in our laboratory, aiming at definitely clarifying this very important aspect of agmatine transport in RBM. The discovery of a transport system for agmatine in RBM accounts for the previous identification of this amine and for its catabolic enzyme, agmatinase, in these organelles (Gorbatyuk et al. 2001). The uptake of agmatine is most probably associated with its effect in preserving and regulating bioenergetic functions and protecting against MPT (Battaglia et al. 2009). The involvement in the regulation of energy production is due to the inhibition of mitochondrial NO-synthase (mtNOS) (Galea et al. 1996) which produces NO which, in turn, inhibits cytochrome C oxidase, with a fall in ATP synthesis (Poderoso et al. 1998). Agmatine transport in RBM, with its protection against bioenergetic collapse as the result of NO action and MPT induction, represents an important safety system against mitochondrial pathology and neurodegeneration.

References

Agostinelli E, Arancia G, Dalla Vedova L, Belli F, Marra M, Salvi M, Toninello A (2004) The biological functions of polyamine oxidation products by amine oxidases: perspectives of clinical applications. Amino Acids 27:347–358

Arndt MA, Battaglia V, Parisi E, Lortie MJ, Isome M, Baskerville C, Pizzo DP, Ientile R, Colombatto S, Toninello A, Satriano J (2009) The arginine metabolite agmatine protects mitochondrial functions and confers resistance to cellular apoptosis. Am J Physiol Cell Physiol 296:1411–1419

Battaglia V, Rossi CA, Colombatto S, Grillo MA, Toninello A (2007) Different behavior of agmatine in liver mitochondria: inducer of oxidative stress or scavenger of reactive oxygen species? Biochim Biophys Acta 1768:1147–1153

Battaglia V, Grancara S, Satriano J, Saccoccio S, Agostinelli E, Toninello A (2009) Agmatine prevents the Ca^{2+}-dependent induction of permeability transition in rat brain mitochondria. In: Agostinelli E (ed) Amino acids (in press)

Cabella C, Gardini G, Corpillo D, Testore G, Bedino S, Solinas SP, Cravanzola C, Vargiu C, Grillo MA, Colombatto S (2001) Transport and metabolism of agmatine in rat hepatocyte cultures. Eur J Biochem 268:940–947

Galea E, Regunathan S, Eliopoulos V, Feinstein DL, Reis DJ (1996) Inhibition of mammalian nitric oxide synthases by agmatine, an endogenous polyamine formed by decarboxylation of arginine. Biochem J 316:247–249

Garlid K, Beavis AD, Ratkje SK (1989) On the nature of ion leaks in energy-transducing membranes. Biochim Biophys Acta 976:109–120

Gorbatyuk OS, Minher TA, Wang G, Regunathan S, Reis DJ (2001) Localization of agmatine in vasopressin and oxytocin neurons of the rat hypothalamic paraventricular and supraoptic nuclei. Exp Neurol 171:235–245

Gornall AG, Bardawill CJ, David MM (1949) Determination of serum proteins by means of the biuret reaction. J Biol Chem 177:751–766

Grillo MA, Colombatto S (2004a) Metabolism and function in animal tissues of agmatine, a biogenic amine formed from arginine. Amino Acids 26:3–8

Grillo MA, Colombatto S (2004b) Arginine revisited: minireview article. Amino Acids 26:345–351

Grillo MA, Battaglia V, Colombatto S, Rossi CA, Simonian AR, Salvi M, Khomutov AR, Toninello A (2007) Inhibition of agmatine transport in liver mitochondria by new charge-deficient agmatine analogues. Biochem Soc Trans 35:401–404

Grundemann D, Hahne C, Berkels R, Schomig E (2003) Agmatine is efficiently transported by non-neuronal monoamine transporters

extraneuronal monoamine transporter (EMT) and organic cation transporter 2 (OCT2). J Pharmacol Exp Ther 304:810–817

Halaris A, Plietz J (2007) Agmatine. Metabolic pathway and spectrum of activity in brain. CNS Drug 21:885–900

Horyn O, Luhovyy B, Lazarow A, Daikhin Y, Nissim I, Yudkoff M, Nissim I (2005) Biosynthesis of agmatine in isolated mitochondria and perfused rat liver: studies with 15N-labelled arginine. Biochem J 388:419–425

Kamo N, Muratsugu M, Hongoh R, Kobatake Y (1979) Membrane potential of mitochondria measured with an electrode sensitive to tetraphenyl phosphonium and relationship between proton electrochemical potential and phosphorylation potential in steady state. J Membr Biol 49:105–121

Klingenberg M (2005) Ligand-protein interaction in biomembrane carriers. The induced transition fit of transport catalysis. Biochemistry 44:8563–8570

Nicholls DG (1978) Calcium transport and proton electrochemical potential gradient in mitochondria from guinea-pig cerebral cortex and rat heart. Biochem J 170:511–522

Palmieri F, Klingenberg M (1979) Direct methods for measuring metabolite transport and distribution in mitochondria. Methods Enzymol 56:279–301

Poderoso JJ, Peralta JG, Lisdero CL, Carreras MC, Radisic M, Schopfer F, Cadenas E, Boveris A (1998) Nitric oxide regulates oxygen uptake and hydrogen peroxide release by the isolated beating rat heart. Am J Physiol 274:112–119

Raasch W, Regunathan S, Li G, Reis DJ (1995) Agmatine, the bacterial amine, is widely distributed in mammalian tissues. J Life Sci 56:2319–2330

Raasch W, Schafer U, Chun J, Dominiak P (2001) Biological significance of agmatine, an endogenous ligand at imidazoline binding sites. Br J Pharm 133:755–780

Regunathan S, Reis DJ (2000) Characterization of arginine decarboxylase in rat brain and liver: distinction from ornithine decarboxylase. J Neurochem 74:2201–2208

Reis DJ, Regunathan S (2000) Is agmatine a novel neurotransmitter in brain? Trends Pharmacol Sci 21:187–193

Rottenberg H (1979) The measurement of membrane potential and ΔpH in cells, organelles, and vesicles. Methods Enzymol 55:547–569

Salvi M, Battaglia V, Mancon M, Colombatto S, Cravanzola C, Calheiros R, Marques MPM, Grillo MA, Toninello A (2006) Agmatine is transported into liver mitochondria by a specific electrophoretic mechanism. Biochem J 396:337–345

Sastre M, Regunathan S, Galea E, Reis DJ (1996) Agmatinase activity in rat brain: a metabolic pathway for the degradation of agmatine. J Neurochem 67:1761–1765

Satriano J, Matsufuji S, Muratami Y, Lortie MJ, Scwartz D, Kelly CJ, Hayashi S, Blantz RC (1998) Agmatine suppresses proliferation by frameshift induction of antizyme and attenuation of cellular polyamine levels. J Biol Chem 273:15313–15316

Satriano J, Kelly CJ, Blantz RC (1999) An emerging role for agmatine. Kidney Int 56:1252–1253

Sener A, Lebrun F, Blaicher F, Malaisse W (1989) Stimulus-secretion coupling of arginine-induced insulin release. Insulinotropic action of agmatine. Biochem Pharmacol 38:327–330

Tesson F, Limon-Boulez I, Urban P, Puype M, Vanderkerkove J, Coupry I, Pompon D, Parini A (1995) Localization of I2-imidazoline binding sites on monoamine oxidases. J Biol Chem 270:9856–9861

Toninello A, Di Lisa F, Siliprandi D, Siliprandi N (1985) Uptake of spermine by rat liver mitochondria and its influence on the transport of phosphate. Biochim Biophys Acta 815:399–404

Toninello A, Miotto G, Siliprandi D, Siliprandi N, Garlid KD (1988) On the mechanism of spermine transport in liver mitochondria. J Biol Chem 263:19407–19411

Toninello A, Dalla Via L, Siliprandi D, Garlid KD (1992) Evidence that spermine, spermidine, and putrescine are transported electrophoretically in mitochondria by a specific polyamine uniporter. J Biol Chem 267:18393–18397

Toninello A, Salvi M, Mondovi B (2004a) Interaction of biologically active amines with mitochondria and their role in the mitochondrial-mediated pathway of apoptosis. Curr Med Chem 11:2349–2374

Toninello A, Salvi M, Pietrangeli P, Mondovì B (2004b) Biogenic amines and apoptosis: minireview article. Amino Acids 26:339–343

Toninello A, Battaglia V, Salvi M, Calheiros R, Marques MPM (2005) Structural characterization of agmatine at physiological conditions. Struct Chem 17:163–175

Vargiu C, Cabella C, Belliardo S, Cravanzola C, Grillo MA, Colombatto S (1999) Agmatine modulates polyamine content in hepatocytes by inducing spermidine/spermine acetyltransferase. Eur J Biochem 259:933–938