Stimulation of choline/Mg\(^{2+}\) antiport in rat erythrocytes by mefloquine

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Abstract. In non Mg\(^{2+}\)-loaded and non malaria-infected rat erythrocytes, mefloquine (100 μmol·l\(^{-1}\)) stimulated choline/Mg\(^{2+}\) antiport without affecting the Na\(^+/\)Mg\(^{2+}\) antiport. The stimulation of the choline/Mg\(^{2+}\) antiport by mefloquine, found in this study, and by trifluoperazine and fluvoxamine, reported previously [Ebel et al. Biochim Biophys Acta 2004; 1167: 132-40], was associated with CF\(_3\) groups attached to the quinoline or benzene ring. The effect of mefloquine on choline/Mg\(^{2+}\) antiport in vitro was not related to the antimalarial action of mefloquine in vivo. In rat erythrocytes, the choline/Mg\(^{2+}\) antiport can be differentiated from the Na\(^+/\)Mg\(^{2+}\) antiport through the use of cinchonine that inhibited the choline/Mg\(^{2+}\) antiport [Ebel et al. Biochim Biophys Acta 2002; 1559: 135-44], and mefloquine that stimulated the choline/Mg\(^{2+}\) antiport, whereby the Na\(^+/\)Mg\(^{2+}\) antiport was not affected by either drug at proper concentrations. The Na\(^+/\)Mg\(^{2+}\) antiport and choline/Mg\(^{2+}\) antiports behave as different molecular entities.

Keywords: Na\(^+/\)Mg\(^{2+}\) antiport, choline/Mg\(^{2+}\) antiport, rat erythrocytes, mefloquine (Lariam\textsuperscript{®}), malaria

In erythrocytes, several types of Mg\(^{2+}\) efflux have been characterized by measuring Mg\(^{2+}\) efflux in different media: Na\(^+/\)Mg\(^{2+}\) antiport in NaCl medium [for a review see 1, 2], choline/Mg\(^{2+}\) antiport in choline-Cl medium [3] and Mg\(^{2+}\) efflux accompanied by Cl\(^{-}\) efflux in sucrose medium [4-6]. Due to the different properties of Na\(^+/\)Mg\(^{2+}\) and choline/Mg\(^{2+}\) antiport they should represent different entities, but to date the molecular structure of these transporters is not known. Thus, differentiation between these possibly different forms of Mg\(^{2+}\) efflux relies on the use of inhibitors.

In rat erythrocytes, both the Na\(^+/\)Mg\(^{2+}\) antiport [6] as well as the choline/Mg\(^{2+}\) antiport [3, 6] can be unspecifically inhibited by amiloride and quinine or quinidine, allowing no differentiation between these two forms of Mg\(^{2+}\) efflux. However, in non Mg\(^{2+}\)-loaded rat erythrocytes, imipramine and other tricyclic drugs carrying a tertiary amine side chain stimulated Na\(^+/\)Mg\(^{2+}\) antiport and inhibited choline/Mg\(^{2+}\) antiport [7], pointing to the transporters as different molecules. Interestingly, cinchonine as another quinoline ring containing compound closely related to quinine, also inhibited the choline/Mg\(^{2+}\) antiport, but had no effect on the Na\(^+/\)Mg\(^{2+}\) antiport [3]. Stimulation of both Na\(^+/\)Mg\(^{2+}\) and choline/Mg\(^{2+}\) antiport could be induced by trifluoperazine and by fluvoxamine [7]. The inhibition of the choline/Mg\(^{2+}\) antiport by tricyclic drugs carrying a tertiary amine side chain was explained by competition of the choline-like side
chain at the choline binding site of the exchanger. The stimulation of the Na⁺/Mg²⁺ antiport and of the choline/Mg²⁺ antiport remained obscure.

In this study we report that mefloquine, a synthetic quinoline derivative stimulated the choline/Mg²⁺ antiport without affecting the Na⁺/Mg²⁺ antiport. The structural requirements for stimulating the choline/Mg²⁺ antiport are discussed by comparing the different drugs.

Materials and methods

Materials
Mefloquine (Lariam®, rac-erythro-α-2-piperidyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol) was a kind gift from Hoffmann-La Roche AG (Grenzach, Switzerland). Nembutal® (pentobarbital sodium) was obtained from Abbott (North Chicago, IL, USA). All other chemicals were purchased at the highest purity available from Merck®, Darmstadt, Germany. Filtered, de-ionized and virtually Mg²⁺-free water with a resistance of 15-18 MΩ/cm was used for the solutions.

Preparation and incubation of red blood cells
The experiments were conducted with non Mg²⁺-loaded rat erythrocytes which have been shown to exhibit a significant Mg²⁺ efflux via the Na⁺/Mg²⁺ antiport [6] and the choline/Mg²⁺ antiport [3] without Mg²⁺ loading. Red cells were prepared as described earlier [7]. In brief, blood (6-8 ml) was consistently obtained from only one anesthetized male Sprague-Dawley rat (50 mg/kg Nembutal® i.p.), weighing 350-450 g. The vena cava inferior was catheterized with a heparinized syringe. Portions of the blood were transferred to heparinized tubes, diluted 1:3 – 1:5 with NaCl medium consisting of 150 mmol·l⁻¹ NaCl, 5 mmol·l⁻¹ D-glucose and 10 mmol·l⁻¹ Hepes-Tris, pH 7.4. The cell suspension was centrifuged at 1000 x g for 10 min at 24 °C. The plasma and the buffy coat containing the white cells were aspirated and discarded. The sedimented red cells were washed twice at 24 °C in NaCl medium. Finally, the red cells were resuspended and incubated with gentle shaking as a 10% (v/v) suspension in one of the following media, each containing 5 mmol·l⁻¹ D-glucose and 10 mmol·l⁻¹ Hepes-Tris, pH 7.4: (a) 150 mmol·l⁻¹ NaCl (NaCl medium), (b) 150 mmol·l⁻¹ choline-Cl (choline-Cl medium). To minimize hemolysis, the cells were handled with utmost caution, the temperature was kept at 24 °C, and centrifugation was carried out at 1000 x g. Paired experiments were consistently performed.

Mg²⁺ efflux
At the beginning of incubation and after 120 min, 1 ml aliquots of the cell suspensions were centrifuged at 1000 x g for 10 min. To determine Mg²⁺, the supernatant was diluted with TCA. The final concentration of TCA was 5% (w/v), containing 0.1% (w/v) La₂O₃ and 0.16% (v/v) HCl. Mg²⁺ was measured in triplicates by means of atomic absorption spectrometry (Perkin Elmer, 2380). Mg²⁺ efflux was calculated from the increase in extracellular Mg²⁺ concentration during the time interval, and was related to the original cell volume measured by hematocrit. Hematocrit was determined by centrifugation at 1500 x g for 10 min. Hemolysis was measured by determining hemoglobin at 557 nm. Mg²⁺ efflux was corrected for hemolysis. For this purpose, Mg²⁺ was extracted from the sedimented erythrocytes with 5% (v/v) TCA and was measured after appropriate dilution of the extract with TCA and La₂O₃–HCl as described above.

Statistical analysis
Data were expressed as mean values ± S.E., and statistical significances were determined by Student’s paired and two tailed t-test. A value of p < 0.05 was considered significant.

Results and discussion
The effect of 100 μmol·l⁻¹ mefloquine on the Na⁺/Mg²⁺ antiport and the choline/Mg²⁺ antiport of non Mg²⁺-loaded and non malaria-infected rat erythrocytes is plotted in figure 1. It can be seen that mefloquine had no effect on the Na⁺/Mg²⁺ antiport but stimulated the choline/Mg²⁺ antiport by approximately 28%. Thus, through structural variants of quinoline derivatives, different effects on the Na⁺/Mg²⁺ antiport and the choline/Mg²⁺ antiport could be obtained: inhibition of both transporters by quinine [3, 6], inhibition of only the choline/Mg²⁺ antiport by cinchonine [3], stimulation of only the choline/Mg²⁺ antiport by mefloquine. This different behavior of Na⁺/Mg²⁺ and choline/Mg²⁺ antiport towards quinoline derivatives can be used as an argument for Na⁺/Mg²⁺ and choline/Mg²⁺ antiport as different molecular entities.

Table 1 lists the structure of several quinoline derivatives and tricyclic compounds that differently affect Na⁺/Mg²⁺ and choline/Mg²⁺ antiports. As concluded earlier, the inhibition of the choline/Mg²⁺ antiport is associated with the choline-like side chain of the
Mg\textsuperscript{2+} efflux as measured in NaCl medium (Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport) and on Mg\textsuperscript{2+} efflux as measured in choline-Cl medium (choline/Mg\textsuperscript{2+} antiport) of non Mg\textsuperscript{2+}-loaded, non malaria-infected rat erythrocytes. Mean values ± S.E., n = 4, *p < 0.05.

Figure 1. Effect of mefloquine (100 µmol·l\textsuperscript{-1}) on Mg\textsuperscript{2+} efflux as measured in NaCl medium (Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport) and on Mg\textsuperscript{2+} efflux as measured in choline-Cl medium (choline/Mg\textsuperscript{2+} antiport) of non Mg\textsuperscript{2+}-loaded, non malaria-infected rat erythrocytes. for choline may be enhanced. At present, a more detailed explanation cannot be given because the structure of the choline/Mg\textsuperscript{2+} antiporter and the exchange mechanism are not known.

Mefloquine is a commonly used antimalarial drug. For a review see [9]. Following infection of human erythrocytes by the malaria parasite Plasmodium falciparum, a NPP of unknown molecular structure is induced that has functional and pharmacological characteristics resembling a volume-regulated anion channel [10-14]. Although the NPP is mainly permeable to anions and neutral solutes, it also allows the permeation of inorganic cations such as Rb\textsuperscript{+}, K\textsuperscript{+}, and Ca\textsuperscript{2+} of the organic cation choline [10, 15-19]. This new choline uptake route via NPP is different from the choline transporter in uninfected human erythrocytes [15].

The mechanism of quinoline-containing antimalarial drugs has not yet been elucidated. The main action seems to be an inhibition of hemoglobin digestion and heme sequestration by the parasite (for a review see [9]). Moreover, in cultured bovine pulmonary artery endothelial cells, an inhibition of the volume-regulated anion channel and of Ca\textsuperscript{2+}-activated Cl\textsuperscript{−}-currents by mefloquine have been reported [20].

Obviously, the stimulation of the choline/Mg\textsuperscript{2+} antiport by mefloquine is not related to its antimalarial action. This is supported by several arguments. In human erythrocytes infected with Plasmodium falciparum, mefloquine did not inhibit the dramatically increased choline uptake via the NPP [19]. In non-infected rat erythrocytes the stimulation of the choline/Mg\textsuperscript{2+} antiport by mefloquine was observed at a drug concentration of 100 µmole·l\textsuperscript{-1}. The IC\textsubscript{50} for inhibition of human intraerythrocyte parasite growth by mefloquine was only 24 nmol·l\textsuperscript{-1} [19], and the therapeutic plasma concentration was found in the range of 1 to 5 µmol·l\textsuperscript{-1} [21, 22]. It should be noted that it is unknown whether rat erythrocytes are less sensitive to mefloquine than human erythrocytes. Furthermore, since choline is a precursor for phospholipid headgroup synthesis of the parasite, inhibition of choline uptake rather than stimulation would be needed to inhibit parasite growth and the production of the tubulovesicular membrane network in the erythrocyte cytosol by the parasite. It is also questionable whether the loss of intracellular Mg\textsuperscript{2+} produced by the stimulation of the choline/Mg\textsuperscript{2+} antiport would suffice to inhibit parasite growth. In in vitro experiments, only severe Mg\textsuperscript{2+} deficiency protected mice against infection with plasmodia which invaded mature erythrocytes [23-26] and in in vitro experiments, the growth of plasmodia was only...
Table 1. Structural requirements for various drugs affecting Mg\(^{2+}\) efflux in NaCl medium (Na\(^{+}\)/Mg\(^{2+}\) antiport) and Mg\(^{2+}\) efflux in choline·Cl medium (choline/Mg\(^{2+}\) antiport) in non Mg\(^{2+}\)-loaded, non malaria-infected rat erythrocytes. Data for imipramine, trifluoperazine and fluvoxamine [7], for quinine and cinchonine [3] were from previous studies by us. Data for mefloquine were from figure 1 of this study. Mg\(^{2+}\) transport is expressed as a percentage difference to the control, with + for stimulation and – for inhibition.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Percent difference</th>
<th>NaCl</th>
<th>Choline·Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipramine</td>
<td><img src="image" alt="Imipramine" /></td>
<td>+32*</td>
<td></td>
<td>-39*</td>
</tr>
<tr>
<td>Quinine</td>
<td><img src="image" alt="Quinine" /></td>
<td>-24*</td>
<td></td>
<td>-62*</td>
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<tr>
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<td>-1 n.s.</td>
<td></td>
<td>-46*</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td><img src="image" alt="Trifluoperazine" /></td>
<td>+12*</td>
<td></td>
<td>+18*</td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td><img src="image" alt="Fluvoxamine" /></td>
<td>+12*</td>
<td></td>
<td>+16*</td>
</tr>
<tr>
<td>Mefloquine</td>
<td><img src="image" alt="Mefloquine" /></td>
<td>-2 n.s.</td>
<td></td>
<td>+28*</td>
</tr>
</tbody>
</table>

*\(p < 0.05\), **n.s.** not significant. The drug concentrations used (nmol·l\(^{-1}\)) were imipramine 0.1, quinine 1.2, cinchonine 1.2, trifluoperazine 0.05, fluvoxamine 0.1, mefloquine 0.1.

Retarded by culturing in Mg\(^{2+}\)-free incubation medium [27, 28].

In summary, in rat erythrocytes, the stimulation of the choline/Mg\(^{2+}\) antiport by the antimalarial drug mefloquine, and the inhibition of the choline/Mg\(^{2+}\) antiport by cinchonidine, reported by us in a previous study [3], can be used for differentiating the choline/Mg\(^{2+}\) antiport from the Na\(^{+}\)/Mg\(^{2+}\) antiport which is not affected by proper concentrations of the drugs. This action of mefloquine on choline/Mg\(^{2+}\) antiport is not related to its antimalarial effect.
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References


