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®), 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.

Abbreviations:

NPP = new permeation pathway

TCA = trichloroacetic acid
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 derivate 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 derivates, 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 derivates 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 derivates 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
tricyclic ring system in imipramine and other tricyclic compounds causing a competitive inhibition of the antiporter [7]. We were not able to identify a structure-function relationship for the inhibition of the choline/Mg2+ antiport by quinine or the structurally related cinchonine, nor for the different effect of both drugs on the Na+/Mg2+ antiport. However, as to the stimulation of the choline/Mg2+ antiport by trifluoperazine and by fluvoxamine, as found in a previous study [7], and by mefloquine as described in this study, the haloalkyl CF3 group attached to the benzene ring might be involved. Mefloquine with two CF3 groups caused a significantly stronger inhibition of the choline/Mg2+ antiport than fluvoxamine with only one CF3 group, when tested at the same drug concentration. There was also a lower effect with trifluoperazine with only one CF3 group. However, to prevent hemolysis, trifluoperazine was tested at a lower concentration which may be compensated by its higher lipophilicity and thus by an increased intercalation into the cell membrane.

The action of the CF3 group(s) can be explained as follows: fluorine is the most electronegative element. The C-F bond with a 44% ionic character is the most ionic of the bonds of carbon with non-metallic elements. The structure of the CF3 group can be described as a resonance hybrid of various structures, containing a C+ atom and one negative charge resonating among the fluorine atoms [8]. When the CF3 groups with these properties are located near the choline/Mg2+ antiporter, the exchange of Mg2+ for choline may be enhanced. At present, a more detailed explanation cannot be given because the structure of the choline/Mg2+ 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+, K+ and 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 Ca2+-activated Cl−-currents by mefloquine have been reported [20].

Obviously, the stimulation of the choline/Mg2+ 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/Mg2+ antiport by mefloquine was observed at a drug concentration of 100 μmol·l−1. The IC50 for inhibition of human intraerythrocyte parasite growth by mefloquine was only 24 nmol·l−1 [19], and the therapeutic plasma concentration was found in the range of 1 to 5 μmol·l−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 Mg2+ produced by the stimulation of the choline/Mg2+ antiport would suffice to inhibit parasite growth. In *in vitro* experiments, only severe Mg2+ deficiency protected mice against infection with plasmodia which invaded mature erythrocytes [23-26] and in *in vitro* experiments, the growth of plasmodia was only

![Figure 1. Effect of mefloquine (100 μmol·l−1) on Mg2+ efflux as measured in NaCl medium (Na+/Mg2+ antiport) and on Mg2+ efflux as measured in choline-Cl medium (choline/Mg2+ antiport) of non Mg2+-loaded, non malaria-infected rat erythrocytes. Mean values ± S.E., n = 4, *p < 0.05.*](image-url)
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 cinchonine, 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.

### 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.

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<th>Drug</th>
<th>Structure</th>
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*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.
Acknowledgments

The skilful and engaged technical assistance of B. Papanis has been greatly appreciated. We are grateful for the kind gift of mefloquine provided to us by Hoffmann-LaRoche AG.

References