Effects of amiloride and furosemide on ^{28}Mg transport into fetuses and maternal tissues of rats

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Zusammenfassung

Nach intraperitonealer Injektion von 16 mg Amilorid oder Furosemid pro trächtige Ratte am 19. Schwangerschaftstag war der Transport von ^{28}Mg in die Feten vermindert. Bei den Muttertieren verursachte Amilorid einen Anstieg des Mg-Gehaltes in Serum, Leber und Nieren, Furosemid erhöhte nur den Mg-Gehalt in den Nieren. Aus den Ergebnissen kann geschlossen werden, daß der Mg-Transport durch die Plazenta durch einen Amilorid-sensitiven Na^+/Mg^{2+}-Antiport und einen Furosemid-sensitiven Mg^{2+}, HCO_3^--Symport bewirkt wird.
Die Zunahme des Mg-Gehaltes in der maternen Leber und Niere unter Amilorid kann dadurch bedingt sein, daß Amilorid den Mg-Efflux stärker hemmt als den Mg-Influx.

Summary

After i.p. injection of 16 mg amiloride or 16 mg furosemide per pregnant rat at day 19 of gestation, ^{28}Mg transport into fetuses was reduced.
In the dams amiloride caused an increase of Mg content in serum, liver and kidney, furosemide only increased Mg content in maternal kidneys.
It is concluded that placental Mg transport is caused by amiloride-sensitive Na^+/Mg^{2+} antiport and by furosemide-sensitive Mg^{2+}, HCO_3^- symport. The increase of Mg content in maternal liver and kidney by amiloride may be caused by a stronger inhibition of Mg efflux than of Mg influx.

Résumé

L'injection intra-péritonéale de 16 mg d'amiloride ou de 16 mg de furosémide chez des rattes au 19ème jour de la gestation a réduit le passage transplacentaire de ^{28}Mg chez le foetus. Chez la mère, l'amiloride a provoqué une augmentation des concentrations sérique, hépatique et rénale de magnésium, alors que sous furosémide l'augmentation de la teneur en magnésium n'a été observée que dans les reins.
Ces résultats permettent de conclure que le passage transplacentaire de magnésium est provoqué par l'«antiport» (couple d'échanges ioniques) Na^+/Mg^{2+} sensible à l'amiloride et par le «symport» (couple de transports iso-directionnels) Mg^{2+}, HCO_3^- sensible au furosémide. L'augmentation de la teneur en Mg, induite par l'amiloride au niveau rénal et hépatique chez la mère, relève peut-être d'une plus forte inhibition de la sortie de Mg que de son entrée.

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Introduction

During fetal development of rats a great amount of magnesium (Mg) is transported to the fetus for the synthesis of fetal body mass which is drastically increased during the last days of gestation.
Moreover, in fetal rats serum Mg concentration is considerably higher than in maternal serum, depending on the developmental state although the concentration of protein, which binds a part of serum Mg, is lower in fetal than maternal serum [11]. The fetal maternal Mg concentration gradient seems to be established by regulation of Mg transport. When maternal serum Mg was increased by i.v. injection of $MgCl_2$ only a very slow increase of fetal serum Mg concentration was found [10].
The mechanism by which Mg is is transported into the fetus or other tissues and its potential regulation are not defined. In experiments with isolated cells we identified a net Mg transport operating via an amiloride-sensitive Na^+/Mg^{2+} antiport (3, 4, 5, 6), and a furosemide-sensitive Mg^{2+}, HCO_3^- symport [7]. In experiments with isolated perfused rat hearts, we found an inhibition of net Mg efflux by amiloride [12].
In experiments with chicken erythrocytes, we found ^{28}Mg influx either due to Na^+/Mg^{2+} antiport under conditions of net Mg efflux or to ^{28}Mg influx in exchange for ^{24}Mg under steady state conditions of Mg distribution. Under both experimental conditions, ^{28}Mg influx was inhibited by amiloride [5]. From these results it was concluded that amiloride can inhibit the same Mg exchanger which under conditions of net Mg efflux is converted to the Na^+/Mg^{2+} antiporter.
Thus, the inhibition of ^{28}Mg influx by amiloride may indicate the existence of such a transport mechanism in a distinct tissue.
Therefore, we tested whether ^{28}Mg influx into fetuses or maternal heart, kidney and liver is inhibited by amiloride or furosemide.

Methods and Materials

Virgin female rats were kept at a 12 h light-dark cycle (light: 9

p.m.–9 a.m.) at about 20 °C. One male rat was caged with 3 females from 6–8 a.m. and impregnated rats were identified by the presence of copulatory plugs. This day was designated as day 0 of gestation.
The rats were fed a normal diet (Mg content: 41 mmol/kg, Ca content 250 mmol/kg) up to day 19 when they were used for the experiments.

1st Experiment

Under Nembutal anesthesia (50 mg/kg s.c.) half of the fetuses were removed from the uteri and blood was taken from the fetal hearts and maternal tail by means of heparinized capillaries and centrifuged for 5 min at 1,300 g. Maternal kidneys were ligated. Three rats were s.c. injected with 8 mg amiloride and 3 rats with 8 mg furosemide per rat dissolved in 0.5 ml dimethylsulfoxide (DMSO). Three rats were injected with 0.5 ml DMSO. Such high drug doses were injected because K_i values for the inhibition of Mg fluxes amounted to 0.3–1 mmol/l (3, 5, 7). Four hours later the remaining fetuses were removed and fetal and maternal serum was taken.
In maternal and fetal serum, Mg concentration was measured by atomic absorption spectrophotometry (AAS, Philipps SP9).

2nd Experiment

At day 19 of gestation 7 pregnant rats were i.p. injected with either 16 mg (53 µmol) amiloride, or 16 mg (48 µmol) furosemide dissolved in 1 ml DMSO. Six control rats were i.p. injected with 1 ml DMSO. Thereafter, each rat was subcutaneously injected with 13.5 µCi $^{28}MgCl_2$, diluted in 0.5 ml 0.15 mol/l NaCl. ^{28}Mg was produced in a cyclotron via the reaction ^{26}Mg (t, p) ^{28}Mg by Dr. *E. Huenges,* Department of Physics, Technical University of Munich, FRG. Specific activity amounted to 21.4 µCi (792 kBq)/µmol Mg. Thus, with the ^{28}Mg activity only 0.63 µmol Mg were injected per rat.
The rats had free access to food and water.
Five hours later, the uteri were removed under Nembutal anesthesia (50 mg/kg s.c.). The fetuses were weighed and directly taken for measurement of ^{28}Mg radioactivity. Maternal blood was taken by heart puncture and centrifuged for 5 min at 1,300 g. Serum was taken for measurement of radioactivity. The hearts, kidneys and aliquots of the livers were removed, cleaned from blood by suspending in cold isotonic sucrose, dotted with filter paper, weighed and taken for measurement of ^{28}Mg radioactivity.
^{28}Mg radioactivity was measured in a gamma-counter (Berthold BF 5300) and corrected for decay of ^{28}Mg.
After measurement of radioactivity all samples were frozen. Three weeks later, the tissues were freeze-dried, ashed in the Plasma Processor 200-E (Technics, GmbH, Munich, FRG). The ash was dissolved in O.1 N HCl and Mg content was measured by AAS.
Maternal serum was deproteinized with 10 % trichloroacetic acid (TCA). Mg content in the TCA extract was measured by AAS.

Results and Discussion

1st experiment

In order to test whether amiloride or furosemide change serum Mg concentration, we injected 8 mg amiloride or 8 mg furosemide in pregnant rats (day 19), their kidney vessels were ligated to prevent renal Mg excretion. Amiloride or furosemide had no significant effect on fetal serum Mg concentration. However, ligation of the kidneys produced doubling of maternal serum Mg concentration already in uninjected rats (Tab. 1). Protein content in serum remained constant (data not shown). To prevent an increase in serum Mg by this procedure, the following experiment with ^{28}Mg was done with rats whose kidneys were intact. In the 2nd experiment fetal serum was not taken to prevent loss of ^{28}Mg from the fetuses.

2nd experiment

General observations
During the 2nd experiment, high doses of amiloride and furosemide had no effects on maternal and fetal body weights. The number of fetuses per litter and the number of resorptions per litter were not significantly changed by amiloride or furosemide (Tab. 2).

Maternal serum Mg concentration
Amiloride caused a drastic increase in maternal serum Mg concentration from 0.8 to 1.3

Tab. 1: Serum Mg concentration (mmol/l) in maternal and fetal rats at day 19 of pregnancy, before and 4 hours after ligation of kidney vessels. Three pregnant rats were injected with 8 mg amiloride or 8 mg furosemide per rat. Three rats served as controls. Mean ± SEM.
Significant differences to serum Mg concentration before ligation of kidney vessels by unpaired Student's t-test b, $p < 0.01$. c, $p < 0.001$.

	maternal serum		fetal serum	
	0 h	4 h	0 h	4 h
Control	0.85 ± 0.08	1.71 ± 0.12[b]	1.58 ± 0.04	1.68 ± 0.09
Amiloride	0.92 ± 0.05	1.91 ± 0.17[b]	1.56 ± 0.05	1.52 ± 0.08
Furosemide	0.94 ± 0.05	1.73 ± 0.05[c]	1.53 ± 0.08	1.49 ± 0.07

mmol/l (Tab. 2). Already a much lower dosis of amiloride (2.5 mg/kg) increased serum Mg concentration [9]. Mg content in maternal tissues was not reduced, but partly increased (see below). Therefore, the increase in maternal serum Mg was not caused by release of intracellular Mg but must have been produced by amiloride inhibition of urinary Mg excretion, as was also found by other authors [9]. In agreement with this effect of amiloride, ^{28}Mg radioactivity in maternal serum was increased to the same ratio as Mg concentration. Thus, the specific activity of ^{28}Mg (cpm/μmol Mg) in maternal serum after amiloride was the same as in the controls.
Furosemide had no significant effect on Mg concentration and ^{28}Mg activity in maternal serum.

Fetal ^{28}Mg uptake
The rate of ^{28}Mg uptake by fetal control rats was the same as was found by other authors [1, 13]. Both drugs decreased ^{28}Mg uptake by rat fetuses (Tab. 2). The increased Mg concentration and ^{28}Mg activity in maternal serum by amiloride can be taken into account by calculating the relative radioactivity (Tab. 3). Thus, because of the increased maternal serum Mg concentration after amiloride injection, the ^{28}Mg transport across the placenta may be more inhibited by amiloride than by furosemide. The amiloride inhibition of fetal ^{28}Mg transport by means of the relative radioactivity (Tab. 3) is more expressed than by the direct data of Tab. 2.
From the well-defined effect of amiloride as an inhibitor of Na^+/Mg^{2+} antiport it can be concluded that the transport of Mg into the fetus is caused by Na^+/Mg^{2+} antiport or at least by an amiloride-sensitive Mg transport because the Na^+ dependence of placental Mg transport cannot be detected in vivo. The furosemide inhibition of fetal ^{28}Mg uptake may indicate the existence of a second transport system which may represent Mg^{2+}, HCO_3^- symport.
An unspecific toxic effect of furosemide on fetal ^{28}Mg uptake may be excluded because in the maternal tissues, furosemide had

Tab. 2.: Body weights, Mg contents, and ^{28}Mg activities in maternal serum and tissues and fetuses under the influence of amiloride and furosemide. Mean ± SEM. Number of pregnant rats in parenthesis. dw, dry weight; ww, wet weight. Significant difference to controls by unpaired Student's t-test. a, $p<0.05$; b, $p<0.01$; c, $p<0.001$.

	Control	Amiloride	Furosemide
Body weight [g]			
Dams	286 ± 9 (6)	276 ± 7 (7)	274 ± 10 (7)
Fetuses	2.3 ± 0.1	2.2 ± 0.1	2.2 ± 0.1
Fetuses/litter	9.5 ± 0.7	8.6 ± 1.3	9.0 ± 2.0
Resorptions/litter	0.8 ± 0.3	1.6 ± 0.6	1.4 ± 0.6
Maternal serum			
Mg [μmol/ml]	0.80 ± 0.03	1.30 ± 0.06[c]	0.90 ± 0.08
^{28}Mg [cpm/ml]	38,868 ± 2,347	66,719 ± 3,864[c]	43,231 ± 5,388
^{28}Mg [cpm/μmol Mg]	48,800 ± 2,240	51,494 ± 2,238	47,643 ± 2,854
Maternal heart			
Mg [μmol/g dw]	46.36 ± 1.29	46.32 ± 0.98	45.70 ± 0.67
^{28}Mg [cpm/g ww]	444,777 ± 20,316	434,740 ± 19,852	432,581 ± 15,491
Maternal kidney			
Mg [μmol/g dw]	35.39 ± 0.63	41.71 ± 0.97[c]	40.56 ± 0.99[c]
^{28}Mg [cpm/g ww]	441,761 ± 51,340	404,023 ± 21,772	426,178 ± 13,728
Maternal liver			
Mg [μmol/g dw]	40.24 ± 0.62	44.91 ± 0.95[b]	41.46 ± 1.96
^{28}Mg [cpm/g ww]	395,517 ± 40,800	551,258 ± 35,622[a]	426,057 ± 11,842
Fetuses			
^{28}Mg [cpm/g ww]	92,593 ± 3,485	77,435 ± 2,210[b]	65,942 ± 7,187[b]

Tab. 3: Relative ^{28}Mg radioactivity $\left(\frac{\text{cpm/g tissue}}{\text{cpm/ml serum}}\right)$ in fetuses and maternal heart, kidney and liver 5 hours after injection of ^{28}Mg and 16 mg amiloride or 16 mg furosemide to pregnant dams at day 19 of gestation. Mean ± SEM. Significant differences to controls by unpaired Student's t-test. b, $p<0.01$; c, $p<0.001$.

	Control	Amiloride	Furosemide
Fetuses	2.38 ± 0.15	1.16 ± 0.09 [c]	1.53 ± 0.19 [b]
Maternal heart	11.44 ± 0.39	6.52 ± 0.46 [c]	10.01 ± 0.77
Maternal kidney	11.37 ± 0.93	6.06 ± 0.22 [c]	9.86 ± 0.97
Maternal liver	11.26 ± 0.66	7.92 ± 0.32 [c]	9.87 ± 0.98

Tab. 4: Specific activity of ^{28}Mg (cpm/μmol Mg) in maternal tissues of rats, 5 hours after injection of ^{28}Mg and 16 mg amiloride or furosemide at day 19 of gestation. Mean ± SEM.

	Control	Amiloride	Furosemide
Heart	42,600 ± 3,001	41,652 ± 1,391	45,143 ± 2,428
Kidney	55,837 ± 6,945	43,322 ± 2,805	55,915 ± 6,414
Liver	31,810 ± 4,236	40,269 ± 2,282	32,579 ± 1,823

no inhibitory effect on intracellular ^{28}Mg uptake (Tab. 2, 3).
The existence of two ^{28}Mg uptake mechanisms may indicate that both mechanisms are localized at the same transporting cells or more probable that the two mechanisms are operating at different cells.

^{28}Mg uptake by maternal tissues
Amiloride did not affect Mg content in maternal heart and increased Mg content in maternal kidney and liver (Tab. 2). Similarly, amiloride also increased Mg content of lymphocytes [9]. So far, the mechanism by which amiloride increases Mg content in these cells has not been defined.
In experiments with isolated hearts, ^{28}Mg influx obeyed Michaelis-Menten kinetics with a K_m value of 0.57 mmol/l (concentration of free Mg^{2+}) [8]. Therefore, taking into account the increased maternal serum Mg, we calculated relative radioactivity for the heart. Suggesting that ^{28}Mg influx into liver and kidney also obeys Michaelis-Menten kinetics, with a K_m value similar to heart, relative radioactivity was calculated for these tissues (Tab. 3).
The values for the relative radioactivity in the controls were in agreement with the values published by *Aikawa* et al [2]. From Tab. 3 it may be concluded that ^{28}Mg uptake by maternal heart, kidney and liver is inhibited by amiloride. However, when the relative specific activity (cpm/μmol Mg) was calculated for maternal tissues (Tab. 4), no significant differences were obtained for controls and amiloride- or furosemide-treated rats.
The potentially controversial results of Tab. 3 and 4 can be explained by the assumption that there is an Mg efflux from maternal tissues, which is more severely inhibited by amiloride than ^{28}Mg transport into the same tissues.

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