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Effects of sodium valproate on magnesium urinary excretion in rats

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Background. Sodium valproate (NaVPA) is known to be increasing diuresis and enhancing the urinary excretion of sodium and chloride ions in rats of both genders. Magnesiuretic effect of NaVPA was not investigated earlier. The aim of the study was to define peculiarities of 24-h urinary magnesium (Mg) excretion in young adult Wistar rats, to evaluate the effect of NaVPA, to reveal gender-related differences in Mg excretion.

Materials and methods. 24-h urinary Mg, creatinine and pH levels were measured in 26 control intact Wistar rats and in 26 Wistar rats after a single intragastric administration of 300 mg/kg NaVPA. 24-h urinary Mg, creatinine and pH levels were also measured in 10 Wistar rats after 10 days of repeated intragastric administration of 300 mg/kg NaVPA and in 12 age-matched control intact Wistar rats.

Results. 24-h diuresis and 24-h Mg excretion were significantly higher in male and female rats after a single as well as after the repeated administration of NaVPA than in gender-matched controls. After a single administration of NaVPA, 24-h urinary Mg excretion per 100 g of body weight was significantly higher in male and female rats than in gender-matched controls, however, after the repeated administration of NaVPA, 24-h urinary Mg excretion per 100 g was significantly higher only in female rats versus controls.

Conclusion. VPA causes magnesiuretic effect with gender-related differences in rats. 24-h magnesiuretic response after administration of the repeated doses of NaVPA in female rats is significantly higher than that in male rats. The mechanism of such gender-related effect is not yet clear.

Key words: valproate, magnesium, urine, rats, gender

INTRODUCTION

Several experimental studies showed effects of biologically active substances on the gender-related transport of ions across cell membrane and differences of ion excretion with urine. Absorption of transepithelial divalent magnesium (Mg) and calcium (Ca) cations in the mice cortical thick ascending limb (TAL) of Henle's loop is an inductive process influenced by gender: irrespective of age, transepithelial Ca and Mg absorption was greater in male than in female mice (1). The greater calciuric response to salt of female salt-sensitive rats versus female salt-resistant rats, which was not seen in an analogous study of male rats, suggests a gender difference in calcium excretion (2). A gender difference in urinary excretion is commonly observed for several organic anions in rats (3). NaVPA, alongside the diuretic effect, enhances K^+ and Cl^- excretion with urine in rats of both genders. The mechanism of this different gender-dependent effect is not clear yet (4).

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The aim of the present study was to evaluate the influence of NaVPA on 24-h urine Mg excretion in Wistar rats of both sexes.

MATERIALS AND METHODS

We examined 26 control intact Wistar rats (13 males and 13 females) and 26 Wistar rats (13 males and 13 females) after a single intragastric administration of 300 mg/kg NaVPA and 12 control intact Wistar rats (6 males and 6 females) and 10 Wistar rats (5 males and 5 females) after 10 days of repeated intragastric administration of 300 mg/kg NaVPA (Convulex, 300 mg/ml drops; Gerot Pharmazeutika, Vienna, Austria) (NaVPA rats). NaVPA dosage was chosen in accordance with the data of preclinical pharmacodynamic studies of NaVPA (5). The experiment, using a single administration of NaVPA, was carried out on age-matched male and female rats. The mean age of control rats was 91 ± 9 days for males and 90 ± 6 days for females. The mean age of NaVPA rats was 97 ± 10 days for males and 95 ± 9 days for females. The mean weight of male rats was 282 ± 31 g in control and 298 ± 23 g in VPA rats. The mean weight of female rats was 238 ± 18 g in control and 240 ± 16 g in VPA rats. The weight was significantly higher in male than in female rats in both groups.

The experiment, using repeated administration of NaVPA, was carried out on age-matched male and female rats. The mean age of control rats was 90 ± 7 days for males and 89 ± 8 days for females. The mean age of NaVPA rats was 96 ± 11 days for males and 98 ± 8 days for females. The mean weight of male rats was 271 ± 29 g in control and 293 ± 27 g in NaVPA rats. The mean weight of female rats was 240 ± 18 g in control and 236 ± 28 g in NaVPA rats. The weight was also significantly higher in male than in female rats in both groups.

The animals were housed in standard colony cages with free access to tap water, the room temperature was 21 ± 1 ºC, and the rats were on a natural light-dark cycle. All experiments were performed according to the institutional guidelines for animal care in order to avoid any unnecessary distress to the animals and to reduce the number of animals used. The animals were housed in described conditions and acclimated for at least 5 days before the experiment. 24-h urine was collected holding a rat alone in a special cage (diuresis cage for rats 3700D000/3701D000, Tecniplast, Italy) for 24 h (from 9:00 a.m. till 9:00 a.m. of the next day) with free access to tap water, without food, in the same temperature and light conditions.

24-h urinary Mg level was analyzed using photometric colorimetric test with a factor for lipid brightening (Magnis liquicolor "Magnesium"). Urinary pH levels were measured with a pH/mV/ion meter (ION Meter pH 340/ION, Germany).

We calculated the 24-h excretion of Mg, creatinine, Mg/creatinine ratio, as well as 24-h diuresis and 24-h urinary Mg excretion per 100 g of body weight.

The data were expressed as means ± SD values from *n* animals. Using Student's test, comparisons between the groups were made. A value of $p < 0.05$ was considered significant. Correlations between two variables were investigated by the method of linear correlation analysis. We applied the Pearson correlation coefficient r, which represents the linear relationship between two variables. A value of $p < 0.05$ was considered significant. We applied STATISTICA for Windows software (StatSoft, USA, 1995) to perform the analysis of our data.

RESULTS

24-h diuresis, 24-h urine Mg level, 24-h Mg excretion, 24-h Mg excretion per 100 g of body weight and Mg/creatinine ratio in control rats showed no statistically significant gender related differences ($p > 0.05$). The 24-h urine pH showed no statistically significant difference ($p > 0.05$) between control female rats (6.57 ± 0.2) and control male rats as well (6.53 ± 0.2).

Diuresis and Mg excretion after a single dose of NaVPA in rats (Table 1). After a single intragastric administration of 300 mg/kg NaVPA, 24-h diuresis was significantly higher in rats of both genders – male and female – versus 24-h urine diuresis of gender-matched control rats ($p < 0.05$), without statistically significant gender related difference ($p > 0.05$).

24-h Mg excretion and 24-h urinary Mg excretion per 100 g of body weight were also significantly higher in NaVPA rats of both genders – male and female – as compared to gender-matched controls ($p < 0.05$), without statistically significant gender related differences ($p > 0.05$).

Mg/creatinine ratio was significantly higher only in NaVPA male rats than in gender-matched control group ($p < 0.05$). 24-h creatinine excretion in NaVPA male rats (0.110 ± 0.025 mmol) was significantly higher ($p < 0.05$) than in NaVPA female rats (0.082 ± 0.017 mmol). Such a difference was not determined between male and female control rats ($p > 0.05$).

The 24-h urine pH in NaVPA males (6.42 ± 0.3) and NaVPA females (6.43 ± 0.3) showed no statistically significant differences versus controls and between genders ($p > 0.05$).

Statistically significant correlations between 24-h Mg excretion and 24-h potassium (K) excretion ($r = 0.70$; $p < 0.05$), between 24-h Mg excretion and 24-h Na excretion ($r = 0.57$; $p < 0.05$) and between 24-h Mg excretion and urine pH ($r = 0.65$; $p < 0.05$) were found in NaVPA males. Only correlation between 24-h Mg excretion and 24-h Na excretion ($r = 0.65$; $p < 0.05$) was statistically significant in NaVPA female rats. Correlations between 24-h Mg excretion and 24-h K excretion ($r = 0.51$; $p > 0.05$) and between 24-h Mg excretion and urine pH ($r = -0.02$; $p > 0.05$) were not statistically significant in NaVPA female rats. No statistically significant correlation between 24-h Mg excretion and 24-h urinary Cl excretion was found in NaVPA male ($r = -0.10$) and female rats ($r = -0.44$; $p > 0.05$).

Diuresis and Mg excretion after repeated doses of NaVPA in rats (Table 2). After 10 days of repeated intragastric administration of 300 mg/kg NaVPA, 24-h diuresis, 24-h Mg excretion were significantly higher in both genders – male and female – than that in gender-matched controls ($p < 0.05$), without statistically significant gender related differences ($p > 0.05$). After the repeated administration of NaVPA, 24-h Mg excretion per 100 g of body weight was significantly higher only in NaVPA female rats ($p < 0.05$) versus gender-matched controls, and 24-h Mg excretion per 100 g of body weight in NaVPA females was significantly higher than that in NaVPA male rats ($p < 0.05$).

Mg/creatinine ratios showed no statistically significant differences between rat groups ($p > 0.05$). The 24-h urine pH

Table 1. **Diuresis and 24-h urinary Mg excretion in male and female control and VPA rat groups (mean ± SD) after a single administration of VPA**

Rat groups	n	24-h diuresis (ml)	24-h Mg level (mmol/l)	24-h Mg excretion (mmol)	24-h Mg excretion per 100 g weight (mmol/100 g)	Mg/creatinine ratio
Control rats						
females	13	8.8 ± 2.1	4.01 ± 0.48	0.035 ± 0.008	0.015 ± 0.004	0.482 ± 0.16
males	13	8.3 ± 2.0	4.26 ± 0.41	0.035 ± 0.008	0.013 ± 0.003	0.429 ± 0.19
VPA rats						
females	13	12.1 ± 4.1•	4.06 ± 0.52	0.049 ± 0.019•	0.021 ± 0.008•*	0.605 ± 0.21
males	13	16.0 ± 7.2•	4.25 ± 0.36	0.067 ± 0.028•	0.023 ± 0.009•*	0.620 ± 0.24•

• statistically significant differences as compared to control group ($p < 0.05$).

* statistically significant differences versus the other gender ($p < 0.05$).

Table 2. **Diuresis and 24-h urinary Mg excretion in male and female control and VPA rat groups (mean ± SD) after repeated administration of VPA**

Rat groups	N	24-h diuresis (ml)	24-h Mg level (mmol/l)	24-h Mg excretion (mmol)	24-h Mg excretion per 100 g weight (mmol/100 g)	Mg/creatinine ratio
Control rats						
females	6	9.0 ± 1.0	3.61 ± 0.35	0.032 ± 0.004	0.014 ± 0.003	0.485 ± 0.16
males	6	7.1 ± 2.0	3.98 ± 0.40	0.028 ± 0.008	0.011 ± 0.004	0.408 ± 0.23
VPA rats						
females	5	11.7 ± 1.8•	3.70 ± 0.34	0.043 ± 0.005•	0.019 ± 0.004•*	0.520 ± 0.15
males	5	10.5 ± 1.7•	3.87 ± 0.65	0.040 ± 0.005•	0.014 ± 0.001•*	0.369 ± 0.12

• statistically significant differences as compared to control group ($p < 0.05$).

* statistically significant differences versus the other gender ($p < 0.05$).

showed no statistically significant difference ($p > 0.05$) among NaVPA females (6.56 ± 0.3), NaVPA males (6.51 ± 0.4) and controls.

The correlations between Mg excretion and Na, K, Cl excretion and urine pH in male and female rats after the repeated doses of NaVPA were not statistically significant ($r < 0.55$; $p > 0.05$).

DISCUSSION

Evidence is increasing indicating that certain drugs could evoke adverse drug effects that depend on the gender of patients. Recent studies have shown that women taking a calcium channel blocker with a diuretic could double their risk of dying from heart disease compared with women using other antihypertensive drug combinations (6). The 24-h magnesiuretic response in female rats to furozemide was significantly higher than in male rats (7). The mechanism of such concomitant effect is not clear. Therefore, elucidation of gender-effect of various drugs by means of preclinical trials is a very important field of pharmacology.

It is known that acute and subacute administration of valproic acid preparations has been shown to exert a moderate diuretic effect on rats (8, 9). A recent study showed that NaVPA, alongside the diuretic effect, enhances gender-related sodium and chloride excretion with urine (4). This difference in excretion of ions with urine might be also related to specific renal haemodynamics caused by NaVPA (7).

The study shows that 24-h diuresis and 24-h Mg excretion, 24-h urinary Mg excretion per 100 g of body weight were significantly higher in male and female rats after a single as well as after the repeated administration of NaVPA than in gender-matched controls. After the repeated administration of NaVPA, 24-h urinary Mg excretion per 100 g was significantly higher only in female rats versus controls. No gender-related differences of 24-h Mg urinary excretion, urinary Mg excretion per 100 g of body weight, urinary pH and diuresis were found in control rat groups.

The research has greatly contributed to our understanding of renal Mg handling. About 80% of total serum Mg is ultrafilterable through the glomerular membrane. Micropuncture experiments, in every species studied to date, indicate that approximately 60% of the filtered Mg is reabsorbed in the loop of Henle, and the superficial distal tubule reabsorbs significant amounts of Mg (10).

The mechanisms of NaVPA increasing sodium, chloride ions excretion, Mg excretion are not clear. Intracellular and extracellular Mg may be an important physiological regulator of sodium and potassium pathways in the cell (11). It was shown that dietary Mg regulates renal thiazide receptor. This relationship between Mg and the thiazide–sensitive Na-Cl co-transport regulating receptor remains to be elucidated (12). Furosemide (Na/K/Cl co-transport inhibitor) might increase Mg excretion by virtue of its effects on the transepithelial voltage thereby inhibiting passive Mg absorption (13). Our data showed that in female rats the 24-h magnesiuretic response to furosemide, when administered 10 mg/kg, was significantly higher than the one in male rats (7). This difference might be also related to gender-specific renal haemodynamics. Female rats have a lower renal haemodynamics as compared to males (14–16). Furthermore, an endothelium-dependent gender-related difference of vascular responsiveness, related to activation of Na/Ca exchanger regulated by Mg, was shown (17). The NaVPA doses applied are known to reduce arterial blood pressure in rats: NaVPA provoked a prolonged cardiovascular depression (8, 18). The mechanism of the cardio depressive effect of NaVPA is not clear; it seems not to involve interference with peripheral vascular noradrenergic activity or arterial baroreflex control (19).

Tissue distribution studies with radiolabeled NaVPA in rodents have shown that NaVPA distributes mainly in the extra cellular space; high levels of radiolabeled NaVPA were found in the liver and kidneys (20). We failed to find data on gender-related differences of NaVPA pharmacokinetics, pharmacodynamics, or gender differences of NaVPA metabolism in rats. Further preclinical and clinical studies elucidating possible mechanisms of NaVPA effects on Mg excretion with urine are necessary.

CONCLUSION

NaVPA alongside increasing diuretic effects in urine also evokes magnesiuric effect in rats of both genders. The mechanism of this different gender-dependent effect is not yet clear. The above reported experimental observations may have potentially important pharmacological implications. Thus, further studies of the mechanisms of NaVPA effects on Mg transport in cells could be important.

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NATRIO VALPROATO POVEIKIS ŽIURKIŲ MAGNIO IŠSISKYRIMUI SU ŠLAPIMU

Santrauka

Įvadas. Nustatyta, kad natrio valproatas didina diurezę ir natrio bei chlorido išsiskyrimą su šlapimu, tuo tarpu natrio valproato poveikis magnio išsiskyrimui su šlapimu netyrinėtas. Eksperimentiniais tyrimais nustatyti su lytimi susiję jonų transporto pro ląstelių membranas, jonų išsiskyrimo su šlapimu skirtumai. Darbo tikslas – nustatyti jaunų subrendusių Wistar žiurkių magnio išsiskyrimo su paros šlapimu ypatumus, įvertinti lyties ir natrio valproato poveikį magnio išsiskyrimui.

Metodai. Magnio ir kreatinino išsiskyrimas su paros šlapimu ir šlapimo pH nustatyti 28 kontrolinėms Wistar žiurkėms ir 26 Wistar žiurkėms po vienkartinės 300 mg/kg natrio valproato dozės, sušvirkštos zondu į skrandį. Magnio ir kreatinino išsiskyrimas su paros šlapimu ir šlapimo pH taip pat nustatyti 10-iai žiurkių po 10 dienų kasdieninių 300 mg/kg natrio valproato dozių, kartą per parą sušvirkštų zondu į skrandį, bei 12-ai atitinkamo amžiaus ir svorio kontrolinių Wistar žiurkių.

Rezultatai. Po vienkartinės ir po kasdieninių natrio valproato dozių patinų ir patelių paros diurezė ir magnio išsiskyrimas su paros šlapimu nustatyti statistiškai patikimai didesni, palyginus su atitinkamos lyties kontrole. Po vienkartinės natrio valproato dozės ir patinų, ir patelių magnio išsiskyrimas su paros šlapimu 100 g kūno svorio buvo patikimai didesnis nei atitinkamos lyties kontrolinių žiurkių, o po kasdieninių natrio valproato dozių tik patelių šis rodiklis buvo patikimai didesnis nei kontrolės.

Išvada. Natrio valproatas didina magnio išsiskyrimą su šlapimu ir šis poveikis patinams ir patelėms yra skirtingas. Kasdieninių natrio valproato dozių minėtas poveikis yra patikimai stipresnis žiurkių patelėms. Su lytimi susijusio šio poveikio mechanizmai dar nežinomi.

Raktažodžiai: valproatas, magnis, šlapimas, žiurkės, lytis