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. The background of relationship between ion metabolism and gender remains to be elucidated. 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).

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 (4). Intracellular and extracellular Mg may be an important physiological regulator of sodium and potassium pathways in the cell (5). However, gender-related differences in Mg transport and Mg excretion remain very poorly studied.

Loop diuretic furosemide (FUR) therapy leads to an increase in urinary and bone losses of Mg (6). It is known that female rats display a lower FUR clearance as a consequence of their lower renal clearance (7). Natriuretic, kaliuretic, and diuretic responses to FUR, when expressed in fractional terms were significantly increased in female rats. This might be explained by a lower abundance of the Na-K-2Cl co-transporter observed in female rats kidneys as compared with male ones (8). The aim of the present study was to evaluate the influence of FUR on gender-related 24-h urine Mg excretion in Wistar rats.

**EXPERIMENT**

We examined 20 Wistar control intact rats (10 males and 10 females) and 12 Wistar rats (6 males and 6 females) after a single intraperitoneal admin-
istration of 10 mg/kg FUR. The experiment was carried out on age-matched male and female rats (mean age 71 ± 6 days) in control group and in FUR group (77 ± 5 days). The mean weight of the male rats was 270 ± 24 g in control and 301 ± 20 g in FUR group and of the female rats 214 ± 25 g in control and 210 ± 27 g in FUR groups. The weight was significantly higher in male than in female rat groups (p < 0.05).

The animals were housed in standard colony cages with free access to food (chow pellets) and tap water. The room temperature was 21 ± 1°C. 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 acclimatized for at least 5 days before experiments. 24-h urine was collected holding a rat alone in a special diuresis cage (diuresis cage for rats 3700D000/3701D000, Tecniplast, Italy) for 24 h (from 8:30 a.m. till 8:30 a.m.) with free access to tap water, without food (under the same temperature and light conditions). In the age-matched rat groups of both gender under the same conditions as for intact control rats the 24-h urine (from 8:30 a.m. till 8:30 a.m.) with free access to food (chow pellets) and tap water. The room temperature was 21 ± 1°C. 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 acclimatized for at least 5 days before experiments. 24-h urine was collected holding a rat alone in a special diuresis cage (diuresis cage for rats 3700D000/3701D000, Tecniplast, Italy) for 24 h (from 8:30 a.m. till 8:30 a.m.) with free access to tap water, without food (under the same temperature and light conditions). In the age-matched rat groups of both gender under the same conditions as for intact control rats the 24-h urine (from 8:30 a.m. till 8:30 a.m.) was collected after a single intraperitoneal administration of FUR in a volume of 10 mg/kg (at 8:30 a.m.).

24-h urinary Mg levels were measured with a spectrophotometer (λ = 520 nm) using special kits for Mg assay (Aqua-Medica, Poland). Sodium and chloride levels were analyzed with an EML-105 electrolyte analyzer (Radiometer, Denmark). Urinary pH levels were measured with a pH/mV/ion meter (ION Meter pH 340/ION, Germany).

We calculated a 24-h excretion of Mg, sodium, chloride and creatinine, a 24-h urinary Mg excretion per 100 g of body weight, and Mg/creatinine ratio. The Mg/creatinine ratio was not used for comparison between genders, as it was found to be a non-informative index while investigating gender-related differences, because creatinine excretion with circadian urine is distinctly higher in male (p < 0.05) than in female rats.

Data were expressed as means ± SD values from n animals. Using Student’s t-test comparisons between groups were made. A value of p < 0.05 was considered significant. Correlations between two variables were investigated by the method of linear correlation. The Pearson correlation coefficient r, was applied, its value was considered significant at p < 0.05.

RESULTS

24-h urinary Mg levels in control males (4.01 ± 0.44 mmol/l) and females (3.80 ± 0.36 mmol/l) showed no statistically significant gender-related difference (p > 0.05). Neither there were any differences (Table 1) in 24-h urine volume (diuresis), Mg excretion and urinary Mg excretion per 100 g of body weight (p > 0.05).

A 24-h diuresis (Table 1) was significantly higher in male (p < 0.02) and female (p < 0.0001) rats after administration of FUR compared with control group. Comparing the diuresis in both gender rats of FUR groups, diuresis was found significantly higher in female than in male rats (p < 0.05).

24-h urinary sodium and chloride excretion as well as sodium and chloride excretion per 100 g of body weight (Table 2) showed no statistically significant gender-related differences in the control groups. After administration of FUR, sodium and chloride excretion per 100 g of body weight in male and female rats (Table 2) was significantly higher than in control male and female rats (p < 0.05), being significantly higher in females than in males (p < 0.05).

24-h urine Mg levels after administration of FUR in males showed no statistically significant differences versus control group (3.79 ± 0.20 mmol/l vs 4.01 ± 0.44 mmol/l; p > 0.05), and in FUR females they were significantly lower than in control female rats (3.20 ± 0.36 mmol/l vs 3.80 ± 0.36; p < 0.02) and significantly lower than in FUR males (3.20 ± 0.36 mmol/l vs 3.79 ± 0.20; p < 0.05). After administration of FUR, 24-h urinary Mg excretion in male and female rats (Table 1) was significantly higher than in control group, without significant gender-related differences. In FUR rat groups Mg excretion per 100 g of body weight in female rats was found significantly higher than in male (p < 0.05) and in control females animals (p < 0.001). Mg excretion per 100 g of body weight in male rats after FUR showed no statistically significant difference versus control males (p > 0.05).

The 24-h urine pH was 6.44 ± 0.2 in control male rats and 6.37 ±0.2 in control female rats. After administration of FUR, the 24-h urine pH was significantly lower in male (6.00 ± 0.19 vs 6.44 ± 0.16; p < 0.05) and female rats (5.95 ± 0.17 vs 6.37 ± 0.23; p < 0.05) as compared to control group. There were no gender-related differences of urine pH in control and FUR groups (p < 0.05) and female rats (5.95 ± 0.17 vs 6.37 ± 0.23; p < 0.05) as compared to control group. There were no gender-related differences of urine pH in control and FUR groups (p < 0.05).
Gender-related magnesium urinary excretion in rats: influence of furosemide.

Data showed no correlation between pH and Mg excretion in control and FUR rat groups.

24-h creatinine excretion was found significantly higher in control male (0.08 ± 0.02 mmol) than in control female rats (0.062 ± 0.01 mmol; p < 0.05). After administration of FUR, 24-h creatinine excretion was found significantly higher in male (0.089 ± 0.01 mol) than in female rats (0.068 ± 0.01 mmol; p < 0.05) too, without statistically significant difference versus control rats. After administration of FUR the Mg/creatinine ratio in female rats (Table 1) was found significantly higher than in control female rats (p < 0.002). The Mg/creatinine ratio in male rats after FUR showed no statistically significant difference from control male rats (p > 0.05).

A correlation between Mg excretion and sodium, between Mg and chloride excretion was not significant in control female and male rats (p > 0.05). In FUR groups, the correlation between Mg excretion and sodium (r = 0.86; p < 0.05) and between Mg and chloride excretion (r = 0.84; p < 0.05) was significant in male rats only.

**DISCUSSION**

There are increasing evidences indicating that certain drugs could evoke adverse drug effects that depend on the gender of patients. The lack of studies on pre-marketing evaluation of drug effects in women was usually explained by ethical reasons. In such situation, theoretically, women are rather unmotivated in the face of certain medicines, and this situation becomes even more serious in the light of new data that women more frequently than men suffer from serious adverse effects. 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 (9). Therefore, elucidation of gender-effect of various drugs is crucial for the development of safer and more effective drugs.

### Table 1. 24-h diuresis and urinary Mg excretion in male and female control and FUR rat groups (mean ± SD).

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>n</th>
<th>24-h diuresis (ml)</th>
<th>24-h Mg excretion (mmol)</th>
<th>24-h Mg excretion per 100 g body weight (mmol/100 g)</th>
<th>Mg/creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>females</td>
<td>10</td>
<td>9.7 ± 2.5</td>
<td>0.037 ± 0.009</td>
<td>0.017 ± 0.005</td>
<td>0.594 ± 0.159</td>
</tr>
<tr>
<td>males</td>
<td>10</td>
<td>10.1 ± 1.8</td>
<td>0.040 ± 0.007</td>
<td>0.015 ± 0.003</td>
<td>0.516 ± 0.092</td>
</tr>
<tr>
<td>FUR group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>females</td>
<td>6</td>
<td>20.2 ± 3.4**</td>
<td>0.064 ± 0.008*</td>
<td>0.031 ± 0.006**</td>
<td>0.955 ± 0.179**</td>
</tr>
<tr>
<td>males</td>
<td>6</td>
<td>14.8 ± 4.2**</td>
<td>0.056 ± 0.013*</td>
<td>0.018 ± 0.004*</td>
<td>0.636 ±0.169**</td>
</tr>
</tbody>
</table>

* = statistically significant difference as compared to control group (p < 0.05);
** = statistically significant differences versus the other gender (p < 0.05).

### Table 2. 24-h urinary sodium and chloride excretion in male and female control and FUR rat groups (mean ± SD).

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>n</th>
<th>24-h sodium excretion (mmol)</th>
<th>24-h sodium excretion per 100 g body weight (mmol/100 g)</th>
<th>24-h chloride excretion (mmol)</th>
<th>24-h chloride excretion per 100 g body weight (mmol/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>females</td>
<td>10</td>
<td>0.188 ± 0.10</td>
<td>0.990 ± 0.05</td>
<td>0.570 ± 0.09</td>
<td>0.275 ± 0.06</td>
</tr>
<tr>
<td>males</td>
<td>10</td>
<td>0.153 ± 0.07</td>
<td>0.056 ± 0.02</td>
<td>0.611 ± 0.23</td>
<td>0.225 ± 0.07</td>
</tr>
<tr>
<td>FUR group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>females</td>
<td>6</td>
<td>0.817 ± 0.06*</td>
<td>0.395 ± 0.06**</td>
<td>1.560 ± 0.20*</td>
<td>0.747 ± 0.08**</td>
</tr>
<tr>
<td>males</td>
<td>6</td>
<td>0.736 ± 0.03*</td>
<td>0.243 ± 0.08**</td>
<td>1.566 ± 0.38*</td>
<td>0.518 ±0.12**</td>
</tr>
</tbody>
</table>

* = statistically significant difference as compared to control group (p < 0.05);
** = statistically significant differences versus the other gender (p < 0.05).
drugs by means of preclinical trials is a very important field of pharmacology.

The present study evaluated the influence of gender and FUR on the 24-h Mg excretion in Wistar rats. Our data showed that 24-h excretions of sodium, chloride, and water were similar in control age-matched intact male and female rats. Compared to male rats, 24-h natriuretic, diuretic and chloriuretic responses to FUR were found significantly higher in females. The mechanism of active NaCl transport in the thick ascending limb (TAL) of Henle’s loop has been elucidated by Gregor and Schlatter who showed that NaCl transport across the apical plasma membrane is mediated by the Na-K-2Cl co-transporter which is directly inhibited by FUR (10). Other investigators showed that the fractional diuretic, natriuretic, and kaliuretic efficiencies of FUR were higher in female rats as compared with males. This might be explained by the lower abundance of the Na-K-2Cl co-transporter observed in homogenates from the medullae of female kidneys as compared with male rats (8). The experiments on rats showed FUR to increase the medulla circulation and sodium excretion, while keeping rats on a standard diet (12). Basal fractional excretion of sodium was significantly lower in male as compared to female rats at similar lower renal perfusion pressure (11).

Infusion of FUR to animals induces structural and functional changes in the distal nephron, including an increase in the activity of the thiazide-sensitive Na-Cl co-transporter (13). The density of the thiazide receptor was twofold higher in females than in males. The increase in the urinary excretion of sodium and chloride caused by thiazide was greater in intact females than in intact males. The renal excretion of sodium and chloride is, in part, controlled via gender-dependent regulation of the renal density of the thiazide diuretic receptor (14). Oestrogen administration has been demonstrated to increase Na-Cl co-transporter protein abundance (14, 15). 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 (16).

FUR, inhibiting the Na-K-2Cl co-transporter and increasing Na, K and Cl excretion with urine, evokes also magnesiuric effect. The mechanism of such concomitant effect is not yet clear. FUR might increase Mg excretion by virtue of its effects on the transepithelial voltage thereby inhibiting passive Mg absorption (17). Our data showed that in female rats the 24-h magnesiuretic response to FUR, when administered 10 mg/kg, was significantly higher than in male rats. This difference might be related also to gender-specific renal hemodynamics. Female rats have a lower renal hemodynamics as compared to male (18, 19, 20). Loop diuretics have a blood pressure-reducing ability independent of blood volume removal (20). FUR caused a reduction in the predominant renal vasoconstrictor effect of the diuretic, which has been reported in rats (22, 23). Furthermore, an endothelium-dependent gender-related difference of vascular responsiveness, related to activation of Na/Ca exchanger regulated by Mg, was shown (24). The Na-Cl co-transporter has been recently demonstrated to be an aldosterone-induced protein (13). A sex-dependent metabolism of aldosterone has been reported in intact rats (25). Additionally, gender differences in renal hemodynamics after FUR might be related to nitric oxide availability, because under baseline conditions male rats have a lower renal nitric oxide synthase activity than female rats (26). Neugarten et al. found that steady state levels of mRNA for inducible nitric oxide synthesis were higher in the inner medulla of female rats as compared with male rats (27). Gender-related tubular absorption and urinary secretion of Mg is important physiological functions for the maintenance of body fluid homeostasis.

CONCLUSION

FUR alongside increasing Na, K and Cl excretion with urine evokes also magnesiuric effect. The 24-h magnesiuretic response in female rats to FUR was significantly higher than in male rats. The mechanism of such concomitant effect is not clear. This difference might be related also to gender-specific renal haemodynamics caused by FUR. Elucidation of these gender-specific mechanisms might be of significance while establishing the risk of adverse effects of drugs in relation to drug-induced ion transport changes in the body. Although additional experimental work must be done in order to bridge the gap between studies using animals and humans, the reported experimental observations may have potentially important pharmacological implications.

REFERENCES

Gender-related magnesium urinary excretion in rats: influence of furosemide.


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