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# ACUTE TOLERANCE DEVELOPMENT TO THE DIURETIC EFFECT OF FUROSEMIDE IN THE RAT

MARGARETA HAMMARLUND AND LENNART K. PAALZOW

*Department of Biopharmaceutics and Pharmacokinetics, University of Uppsala, Sweden*

## ABSTRACT

Furosemide was given to rats as five different i.v. bolus doses (2·5–100 mg $kg^{-1}$), or as an i.v. infusion to a steady-state concentration in plasma of 14 μg $ml^{-1}$. The urinary furosemide excretion rate ($\Delta Ae/\Delta t$) and the diuretic effect (volume of urine) were measured. A parallel shift in the excretion–response curve was seen as a fivefold increase in $(\Delta Ae/\Delta t)_{50}$ ($(\Delta Ae/\Delta t)$ at half-maximal effect) between the i.v. bolus doses from 2·5 to 40 mg $kg^{-1}$. The slope factor did not change. During infusion, a decrease in efficiency to 20 per cent of the initial value was seen. These results are indicative of an acute tolerance development to the diuretic effect of furosemide. Some intrarenal feedback inhibition mechanism might be involved, as the extracellular fluid volume seems to be of great importance to the effect obtained. The resulting effect can be compared with the influence of a competitive antagonist.

KEY WORDS Furosemide Acute tolerance development Pharmacokinetics Pharmacodynamics Efficiency

## INTRODUCTION

In recent years, many important contributions have been made to the understanding of the pharmacokinetics and pharmacodynamics of furosemide. Furosemide, given with and without probenecid, has, for example, revealed the urinary excretion of furosemide to be more closely related to the effect than is the plasma concentration.[1–7] Furosemide is mainly secreted via the active secretory pathway in the proximal tubule,[8, 9] and the effect is elicited from the luminal side of the nephron by an inhibition of the chloride transport in the ascending limb of the loop of Henle.[10] The urine can thus be considered to be closest to the site of action of furosemide.

Many authors found a discrepancy between the obtained overall effect of

Requests for reprints should be sent to: Dr. Margareta Hammarlund, Department of Biopharmaceutics and Pharmacokinetics, University of Uppsala, Biomedical Center, Box 580, S-751 23 Uppsala, Sweden.

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furosemide and its urinary excretion, depending on the mode of administration,[11–13] or as a consequence of probenecid pretreatment.[3, 5, 14] Kaojaren *et al.*[15] discuss the time course of the delivery of furosemide to the site of action as an independent determinant of the overall response. However, this does not seem to be the only explanation of the discrepancies found. Electrolyte and volume status also seems to have an influence on the obtained effect[14, 16] which together with the time course of the furosemide delivery into the urine seems to influence the development of an acute tolerance to the effect. This has been studied in the present paper in the rat and elsewhere[13] in humans.

## MATERIALS AND METHODS

### *Animals*

Male Sprague–Dawley rats were used throughout the study. They weighed $200 \pm 28$ g (i.v. bolus dose study, $n = 28$), and $229 \pm 46$ g (infusion study, $n = 7$).

Two days before the administration of furosemide two silicon rubber cannulae were implanted under light ether anaesthesia into the jugular veins of the rat. The urinary bladder was cannulated with a catheter via the abdomen. The urinary as well as the venous catheters were exteriorized through the abdomen via a metal fistula extending about 6 cm outside the skin. A more thorough description of the surgical preparation is presented in Reference 17.

The rat was placed in a cage with a track through which the fistula was protruded. The rat could move freely along the track. Urine samples were collected directly into small plastic vials attached to the fistula under the cage. This arrangement permitted exact administration of furosemide in one venous catheter and blood sampling in the other. It also made it possible to take blood and urine samples at exact points of time without stressing the rat.

The day before the experiment the rat was allowed to rest and normal urinary output was measured. The urine samples were weighed as a measure of the urinary output.

### *Intravenous bolus doses*

Furosemide was given as i.v. bolus doses of 2·5, 10, 20, 40, and 100 mg $kg^{-1}$. The urine samples were collected at 5, 10, 15, 20, 25, 35, 50, 70, and 180 minutes. The number of animals in each dose group were 6 (2·5–20 mg $kg^{-1}$) and 5 (40–100 mg $kg^{-1}$), respectively. The rats had free access to food and water during the experiment. No other attempt was made to compensate for fluid losses.

### *Constant intravenous infusion*

Constant infusion of furosemide was given to 7 rats. The infusion was given via one of the venous catheters for 6 hours. To obtain the desired steady-state plasma concentration level as quickly as possible, the technique with two consecutive infusion rates was used.[18] The first rapid infusion was given at the

rate of 280 μg min$^{-1}$ kg$^{-1}$ ($Q_1$) during the first 15 minutes. Then (from 15 to 360 minutes) the infusion rate was reduced to 67 μg min$^{-1}$ kg$^{-1}$ ($Q_2$). These rates were calculated to give a steady-state concentration in plasma of 10 μg ml$^{-1}$. The calculations were based on the parameter values obtained from the administration of 40 mg kg$^{-1}$.[17] A volume of about 0·4 ml min$^{-1}$ was infused during the first 15 minutes, thereafter 0·10 ml min$^{-1}$. The total volume infused was about 41 ml. The rats had free access to food and water during the study.

Urine was collected every 15 minutes during the first 2 hours, then every 30 minutes. Plasma samples were withdrawn via the other venous catheter at 135, 255, and 345 minutes.

*Chemical assay*

Furosemide concentration were determined in plasma and urine with HPLC with UV detection at 280 nm. The samples were extracted from the acidified water phase with diethyl ether as described before.[17]

*Data analysis*

The effect model was fitted to the experimental data by the non-linear least square regression programs NONLIN and DARE-MINUIT.[19] The data points were given equal weights. Several runs with different initial estimates were performed to avoid local minima in the sum of square surfaces. Significance for and between data was obtained with conventional statistical methods such as linear regression, analysis of variance, and $t$-test. The goodness of fit of computed data to observed data was based on visual inspection, coefficient of correlation ($r$), coefficient of determination ($r^2$), and standard deviation of parameters. All values are presented ±S.D. if not otherwise stated.

*Calculations*

*Infusion rates.* The two consecutive infusion rates were calculated from the following equations:[18]

$$Q_2 = \mathrm{Cl} \cdot C_{ss} = \frac{\text{dose}}{\text{AUC}} \cdot C_{ss} \tag{1}$$

$$Q_1 = Q_2/(1 - e^{-\gamma \cdot T}) \tag{2}$$

where $Q_1$ and $Q_2$ are the rapid and the slow infusion rates, respectively. Cl is total plasma clearance and $C_{ss}$ is the desired steady-state plasma concentration in the central compartment (plasma). The values of the area under the curve (AUC), the dose, and the disposition rate constant of the last phase ($\gamma$) were respectively 6039 μg min$^{-1}$ ml$^{-1}$, 9087 μg, and 0·0141 min$^{-1}$ (dose 40 mg kg$^{-1}$, Reference 17). $T$ is the time during which the infusion is given at the rapid rate $Q_1$, here 15 minutes.

*Other pharmacokinetic calculations*

Total clearance, Cl:

$$Cl = \text{dose}/AUC_{\infty} \tag{3}$$

or

$$Cl = k_0/C_{ss} \tag{4}$$

where $k_0$ is the infusion rate.

Renal clearance, $Cl_r$:

$$Cl_r = f_e \cdot Cl \tag{5}$$

or

$$Cl_r = (\Delta Ae/\Delta t)/C_{ss} \tag{6}$$

$$f_e = (\Delta Ae/\Delta t)/k_0 \quad \text{at plateau} \tag{7}$$

where $f_e$ is the fraction of the dose excreted unchanged into the urine, and $\Delta Ae/\Delta t$ is the urinary excretion rate of furosemide.

*Effect modelling*

The effect is measured as the urine flow rate (ml min$^{-1}$). The relationship between the urinary excretion rate of furosemide ($\Delta Ae/\Delta t$, μg min$^{-1}$), and the effect ($E$), was evaluated using the Hill equation:

$$E = \frac{E_{max} \cdot (\Delta Ae/\Delta t)^S}{(\Delta Ae/\Delta t)^S + (\Delta Ae/\Delta t)^S_{50}} + E_0 \tag{8}$$

$E_{max}$ is the maximal effect, 0·28 ml min$^{-1}$ (Figure 1). $(\Delta Ae/\Delta t)_{50}$ is a constant representing the furosemide excretion rate at half-maximal effect. $E_0$ is the normal urine flow rate, 0·011 ml min$^{-1}$. $S$ is the slope factor.

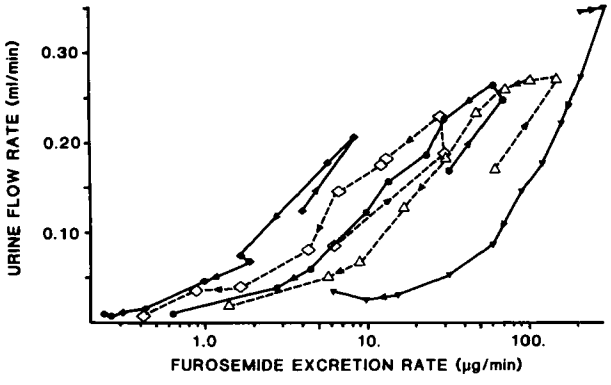


Figure 1. Excretion–response relationships for furosemide after i.v. bolus doses given to rats. The parallel shift in the curves indicates tolerance development. Counter-clockwise hysteresis can also be seen for some of the doses. (◆: 2·5,◇: 10, ●: 20, △: 40, and ▼: 100 mg kg$^{-1}$, respectively. Mean values)

*Efficiency*

The efficiency of furosemide (ml $\mu g^{-1}$) is calculated as the increase in the urine flow rate per renal excretion rate of furosemide, and is used to evaluate the excretion–response relationship:

$$\text{Efficiency} = \frac{E - E_0}{(\Delta Ae/\Delta t)} \tag{9}$$

The overall efficiency is measured as the total volume of urine excreted divided by the total amount of furosemide excreted.

## RESULTS

*Intravenous bolus dose*

The basal urine flow rate ($E_0$) was $0{\cdot}011 \pm 0{\cdot}007$ ml $min^{-1}$ ($n = 28$) for all the rats given i.v. bolus doses of furosemide (N.S. between dose groups). The fraction of furosemide excreted unchanged into the urine increased from 30 per cent to 38 per cent as the dose was increased from 2·5 to 100 mg $kg^{-1}$ (Table 1, see also Figure 3 in Reference 18). The increase was significant.[17] Average renal clearance was 0·36 ml $min^{-1}$ and 0·52 ml $min^{-1}$ for 10 and 40 mg $kg^{-1}$, respectively, whereas total plasma clearance was 1·2 and 1·5 ml $min^{-1}$.[17] The cumulative effect (urine volume) up to 180 minutes, and the cumulative amount of furosemide excreted unchanged after the i.v. bolus doses, can also be seen in Table 1.

Table 1. Cumulative effect, cumulative amount of furosemide excreted up to 180 minutes, fraction of the dose excreted unchanged, and overall efficiency after intravenous bolus doses of furosemide to rats. Mean values ± S.D.

| Dose (mg $kg^{-1}$) | Cumulative effect ($E-E_0$, ml) | Cumulative amount of furosemide excreted unchanged (μg) | Fraction of the dose excreted unchanged | Overall efficiency (ml $\mu g^{-1}$) |
|---|---|---|---|---|
| 2·5 | 3·4 ± 0·5 | 154 ± 30 | 0·299 ± 0·066 | 0·0222 ± 0·003 |
| 10 | 7·3 ± 1·2 | 670 ± 170 | 0·310 ± 0·069 | 0·0114 ± 0·003 |
| 20 | 10·0 ± 2·1 | 1500 ± 313 | 0·335 ± 0·064 | 0·0070 ± 0·003 |
| 40 | 12·5 ± 3·2 | 2640 ± 727 | 0·344 ± 0·088 | 0·0042 ± 0·002 |
| 100 | 11·9 ± 4·1 | 8400 ± 1300 | 0·381 ± 0·034 | 0·0014 ± 0·0003 |

The excretion–response curves for the five different doses given are shown in Figure 1. The Hill equation, equation (8), was fitted to the data. In the calculations, the experimentally obtained maximal and minimal effects of 0·28 and 0·011 ml $min^{-1}$, respectively, were used. With increasing doses a parallel shift to the right can be seen, while the maximal effect is of the same magnitude

for the four lowest doses. A good correlation to the experimental values was accomplished, and is plotted for the individual doses in Figure 2(a)–(d). For the 100 mg kg$^{-1}$ dose, a maximal effect of 0·35 ml min$^{-1}$ was observed. As no plateau was seen in the curve (Figure 2(e)), calculations were performed to estimate the $E_{max}$ value together with the estimations of $S$ and $(\Delta Ae/\Delta t)_{50}$ (equation (8)) (Table 2).

Table 2. Calculated values for slope factor ($S$) and furosemide excretion rate at half-maximal effect $(\Delta A_e/\Delta t)_{50}$, and given or calculated values of $E_{max}$. $E_0$ was 0·011 ml min$^{-1}$. Mean values ± S.E.E.

| Dose (mg kg$^{-1}$) | $S$ | $(\Delta Ae/\Delta t)_{50}$ (μg min$^{-1}$) | $E_{max}$ (ml min$^{-1}$) | $r^2$ |
|---|---|---|---|---|
| 2·5 | 1·47 ± 0·07 | 4·17 ± 0·17 | 0·28 | 0·993 |
| 10 | 1·44 ± 0·14 | 7·95 ± 0·50 | 0·28 | 0·981 |
| 20 | 1·32 ± 0·09 | 13·2 ± 0·6 | 0·28 | 0·991 |
| 40 | 1·59 ± 0·06 | 20·5 ± 0·6 | 0·28 | 0·998 |
| 100 | 1·91 ± 0·23 | 82·5 ± 5·3 | 0·28 | 0·978 |
| 100 | 1·21 ± 0·13 | 298·0 ± 17·0 | 0·733 ± 0·027 | 0·970 |

The parameter values obtained for the slope factor $S$ and the furosemide excretion rate at half-maximal effect, $(\Delta Ae/\Delta t)_{50}$, can be seen in Table 2. No clear trend could be found in the values of the slope factor $S$, which varied between 1·3 and 1·6 (1·9 or 1·2 for 100 mg kg$^{-1}$, depending on the $E_{max}$ value). The constant $(\Delta Ae/\Delta t)_{50}$ increased dramatically with dose, as can also be seen in the parallel shift of the excretion–response curves in Figure 1. During the first collection period (0–5 minutes), a tendency to a counter-clockwise hysteresis can be seen for 10, 20, and 40 mg kg$^{-1}$, indicating a small time-delay between the furosemide excretion rate and the effect. In the fit of the Hill equation, this first value(s) was not considered (in parentheses in Figure 2).

The individual values of the overall efficiency for each rat were calculated according to equation (9) and are presented in Figure 3, plotted against the logarithm of the cumulative amount of furosemide excreted. A drastic linear decrease in efficiency can be seen with the increasing amount of furosemide excreted ($F = 285$, $p < 0·0001$, $r^2 = 0·92$). The dose 40 mg kg$^{-1}$ is thus only one-fifth as efficient as 2·5 mg kg$^{-1}$. Although 100 mg kg$^{-1}$ gives a higher initial maximal effect than the other doses, the overall efficiency is markedly reduced. The mean value of the overall efficiency for each dose is presented in Table 1.

*Constant intravenous infusion*

The basal urine flow rate during the day before the experiment was 0·011 ± 0·0003 ml min$^{-1}$ for the 7 rats given constant infusion.

From the infusion rates given (equations (1)–(2)), and considering the weights of the rats, and, based on the pharmacokinetic data from the 40 mg kg$^{-1}$ dose,[17]

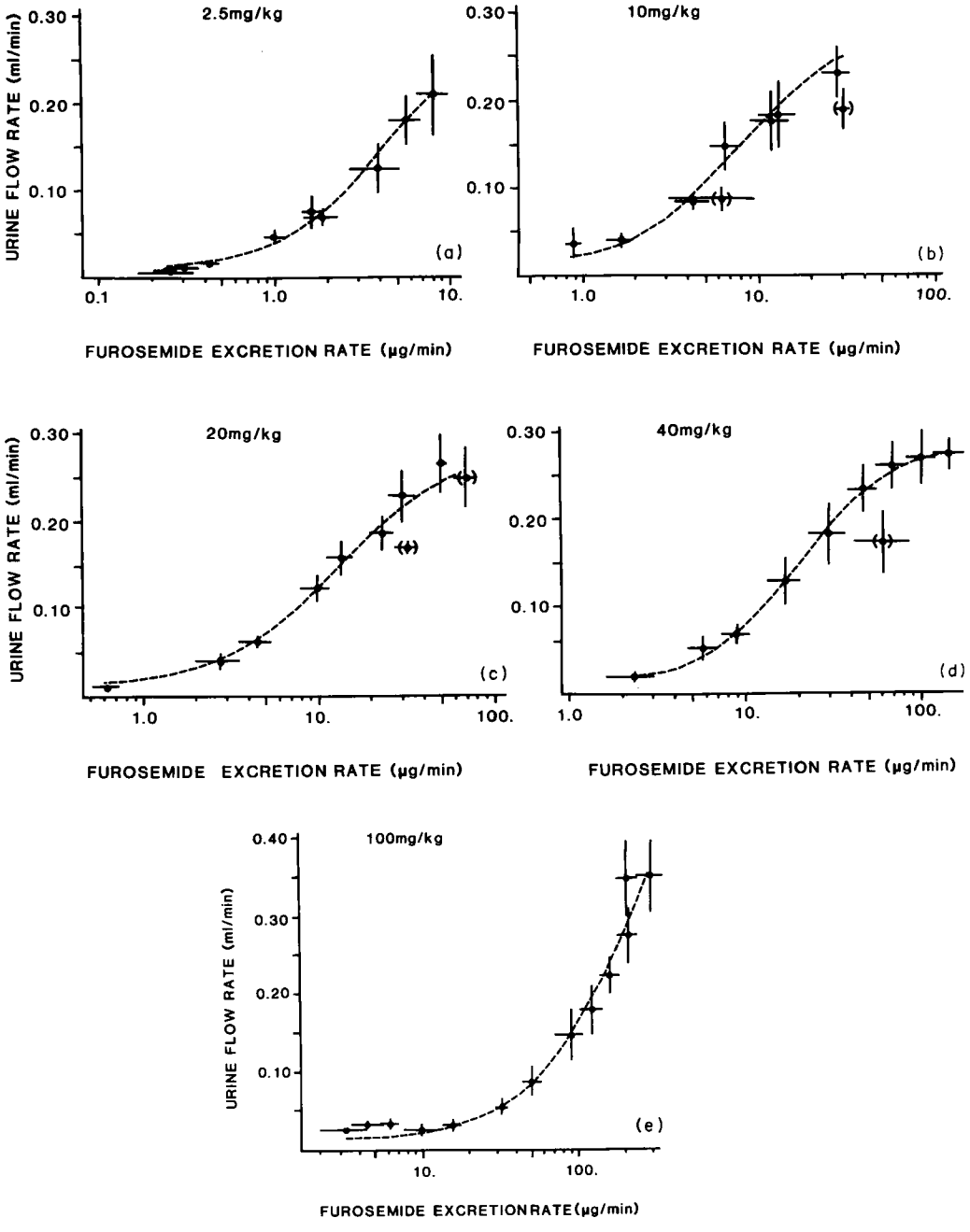


Figure 2(a)–(e). Excretion–response relationships for the different i.v. bolus doses of furosemide given to rats. The lines represent the fit of the Hill equation to the data (except for the data points within parentheses). Mean values ± S.E.M.

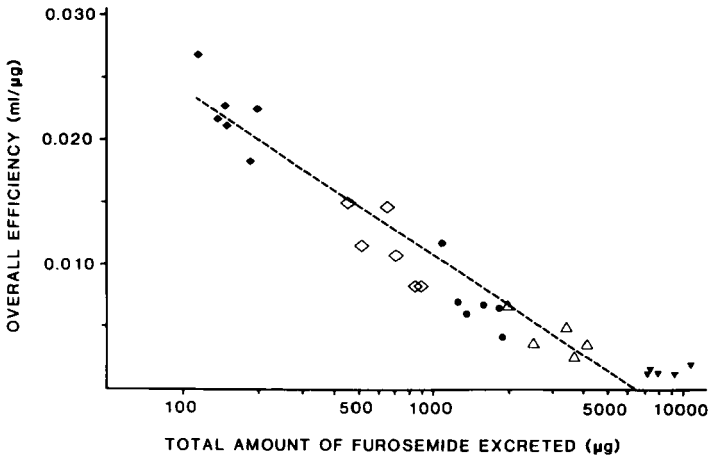


Figure 3. Overall efficiency (individual values for each rat) vs. the cumulative amount of furosemide excreted (180 minutes) after i.v. bolus doses. Linear regression (dotted line) gave $r^2 = 0{\cdot}92$ and $p < 0{\cdot}0001$. (The dose groups are the same as in Figure 1)

a calculated plasma concentration at a steady state of $12 \pm 3\,\mu g\,ml^{-1}$ would be reached. In Table 3, the values obtained for $C_{ss}$ for the sampling times 135, 255, and 345 minutes can be seen. No significant difference in $C_{ss}$ was found between these times, even if there was a slight continuous increase. A mean value for all measurements was calculated to be $14 \pm 7\,\mu g\,ml^{-1}$. For the clearance values (Table 3), there was no significant difference between the three sampling times. Thus, the average total plasma clearance for furosemide was $1{\cdot}0 \pm 0{\cdot}4\,ml\,min^{-1}$ (equation (4)). The renal clearance was $0{\cdot}36 \pm 0{\cdot}15\,ml\,min^{-1}$ (equation (6)). The fraction excreted unchanged during steady state was $0{\cdot}39 \pm 0{\cdot}15$ (equation (7)).

The furosemide excretion rate and the urine flow rate during the 6 hours are shown in Figure 4. While the furosemide excretion rate was mainly stable or somewhat increasing, the effect was gradually decreasing after the start of the infusion. The calculated efficiency in each sampling interval (equation (9)) can be

Table 3. Plasma concentrations ($C_{ss}$), total (Cl) and renal clearance ($Cl_r$) at steady state during constant infusion of furosemide to rats. (Theoretical value for $C_{ss}$ was $12{\cdot}5 \pm 2{\cdot}8\,\mu g\,ml^{-1}$)

| Sampling interval (minutes) | $n$ | $C_{ss}$ ($\mu g\,ml^{-1}$) | Cl ($ml\,min^{-1}$) | $Cl_r$ ($ml\,min^{-1}$) |
|---|---|---|---|---|
| 120–150 | 7 | $13{\cdot}3 \pm 6{\cdot}5$ | $1{\cdot}18 \pm 0{\cdot}39$ | $0{\cdot}347 \pm 0{\cdot}146$ |
| 240–270 | 7 | $14{\cdot}9 \pm 6{\cdot}8$ | $0{\cdot}80 \pm 0{\cdot}33$ | $0{\cdot}385 \pm 0{\cdot}176$ |
| 330–360 | 7 | $15{\cdot}1 \pm 8{\cdot}6$ | $1{\cdot}02 \pm 0{\cdot}50$ | $0{\cdot}350 \pm 0{\cdot}152$ |
| 120–360 | 21 | $14{\cdot}4 \pm 7{\cdot}0$ | $1{\cdot}00 \pm 0{\cdot}42$ | $0{\cdot}361 \pm 0{\cdot}150$ |

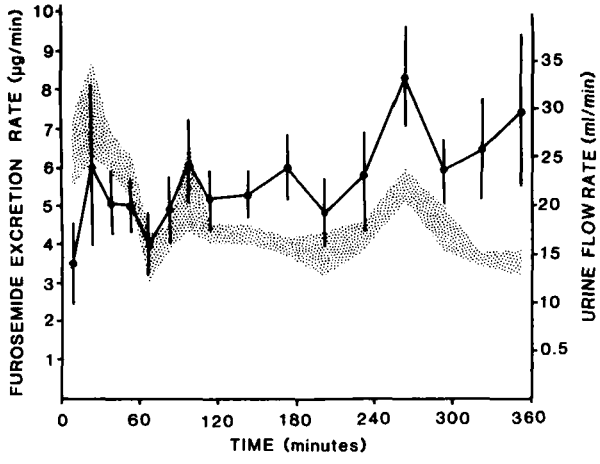


Figure 4. Furosemide excretion rate (straight line with mean values ± S.E.M.) and urine flow rate (dotted area covering the range ± S.E.M.) after constant intravenous infusion to rats

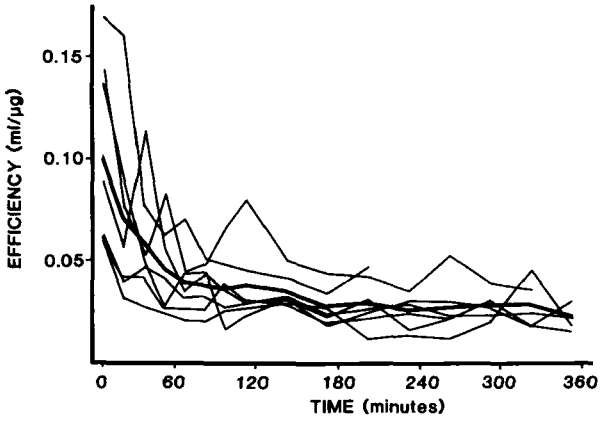


Figure 5. Efficiency during constant intravenous infusion to rats. The narrow lines show the individual values for each rat, while the wider line shows the mean value in each collection interval

seen in Figure 5. After the start of the infusion the efficiency of the diuretic effect decreased slowly from 0·10 ml $\mu g^{-1}$ at 0–15 minutes to a final efficiency of 0·02 ml $\mu g^{-1}$ at 330–360 minutes, a decrease to 20 per cent. Selected values are presented in Table 4.

## DISCUSSION

It is well established that furosemide exerts its effect from the luminal side of the nephron. Many authors[1–7] have also reported a closer relationship for the

Table 4. Results obtained after constant infusion of furosemide to rats

| Time (mid-point, minutes) | Cumulative volume excreted (ml) | Cumulative volume infused (ml) | Cumulative amount of furosemide excreted (μg) | Efficiency in the sampling interval (ml μg$^{-1}$) |
|---|---|---|---|---|
| 7·5 | 3·7 ± 1·5 | 6·6 | 38·5 ± 22·9 | 0·103 ± 0·096 |
| 22·5 | 7·6 ± 2·4 | 8·1 | 100 ± 53 | 0·071 ± 0·044 |
| 47·5 | 14·7 ± 3·1 | 11·1 | 238 ± 93 | 0·046 ± 0·022 |
| 112·5 | 25·0 ± 3·7 | 17·1 | 512 ± 134 | 0·038 ± 0·020 |
| 165 | 35·0 ± 4·2 | 23·1 | 832 ± 193 | 0·027 ± 0·009 |
| 345 | 65·8 ± 3·4 | 41·1 | 1978 ± 597 | 0·022 ± 0·005 |

pharmacodynamic response to the urinary furosemide excretion rate, rather than to the plasma concentration. Even though the tubule is considered the site of action, a small counter-clockwise hysteresis indicating a distributional delay can be seen for some of the doses in the present study. This has also been found in humans after i.v. administration[13] and in dogs after bumetanide administration.[20] In the hen, however, there was a minute by minute correlation between the furosemide excretion and the effect.[4]

A previous paper[17] presented the pharmacokinetic behaviour of furosemide in the rat after i.v. bolus doses. In parallel to this study, the diuretic effect was measured as the volume of urine excreted per unit of time. In the rats, we found an increased renal clearance of furosemide with increasing plasma concentrations of the drug. The main reason for this finding is probably an increased free fraction of furosemide in plasma, caused by a saturable protein binding.[17] Thus, the renal excretion of the drug increases more than proportionally with increasing plasma concentrations.

In contrast to the increased renal clearance, the present investigation showed a relative decrease in the effect of furosemide with increasing dose, as indicated by the parallel shift to the right in the excretion–response curves with increasing dose. This behaviour is a classical indication of tolerance development, i.e. a decreased potency with increasing dose. In the fit of the Hill equation to the data, this was seen as a fivefold increase in $(\Delta Ae/\Delta t)_{50}$ (furosemide excretion rate producing half-maximal effect) between the 2·5 and the 40 mg kg$^{-1}$ dose (Table 2, Figures 1 and 2). The 100 mg kg$^{-1}$ dose deviates from the rest in the shape of the excretion–response curve with a higher maximal effect, eventually because it is a supermaximal dose. No clear trend was found in the value of the slope factor $S$.

The same slope factor and the same maximal effect are typical of different drugs that produce the same effect by the same mechanism, but differ in potency. This also applies to a drug given with or without a competitive antagonist. Thus, we can make a comparison with the present study. Except for the 100 mg kg$^{-1}$

dose, the effect mechanism itself for furosemide does not seem to be influenced (same $E_{max}$). Instead some kind of mechanism is induced that behaves like a competitive antagonist, shifting the curve to the right. The exact meaning of such an 'endogenous antagonism' is not clear at the present time. It might be some kind of physiological feedback inhibition of the effect. This 'endogenous antagonism' is obviously developed very quickly after i.v. bolus administration, since already shortly after administration the excretion–response curves show this parallel shift. Branch *et al.*[16] have also discussed the shift in concentration–response curves as being some competitive inhibition.

When furosemide is administered much more slowly, and thus is presented to the site of action more slowly, the development of tolerance should perhaps be revealed. Thus, by giving i.v. infusions of furosemide to rats, and establishing a steady-state plasma concentration, a continuous change in the diuretic effect would be evidence of tolerance development. This was also seen. Despite a relatively constant urinary excretion rate of furosemide, the urine flow rate decreased gradually (Figure 4). When presented as efficiency, the development of tolerance to the diuretic effect is evident, with a gradual decrease of the efficiency to 20 per cent of the first measurement (Table 4, Figure 5). The overall efficiencies from the i.v. bolus data also showed a drastic decrease with increasing dose (Figure 3). In fact, $40\,mg\,kg^{-1}$ gave only 20 per cent of the efficiency in comparison with $2{\cdot}5\,mg\,kg^{-1}$. In our study in humans[13] we could also detect a tolerance development as a clockwise hysteresis, when furosemide was administered orally after food intake. Thus, the development of tolerance can be detected both in the rat and in humans, when furosemide is given slowly.

In the present study, the fluid balance in the rat was negative, as we did not fully compensate for the fluid losses. This is a condition that most probably influences the obtained diuretic effect. Our finding in this study and in the one in humans[13] is supported by others. Homeida *et al.*[14] found a decrease in the ratio of sodium to furosemide excretion that diminished when furosemide was given in a higher dose without fluid compensation, or when it was given to sodium-depleted subjects. Branch *et al.*[16] also observed a change in the plasma concentration–response curve for furosemide with a slight shift to the right of the curve for subjects having received furosemide 36 hours earlier. The weakness of their study in this respect was that they only compared the plasma concentrations, not the urinary excretion rate with the effect. In a study of bumetanide in the dog, Smith *et al.*[20] showed that pretreatment with probenecid shifts the excretion–response curve to the left, which gives higher efficiency. Thus, a somewhat smaller amount of bumetanide is excreted within the first 40 minutes after probenecid, without a concomitant decrease in the response. In the light of the present study, one explanation might be an acute tolerance development also to the effect of bumetanide. In the urine of nephrotic rats, Green *et al.*[21] have shown a moderating influence of the protein binding of furosemide on the diuretic effect. However, although not discussed, the water-deprived rats in the same study show an even further shift to the right in the

excretion–response curve in comparison with the control. Volume status thus seems to be one of the most powerful determinants of the diuretic effect, even more so than the protein binding in the urine. The observations on volume depletion are in good agreement with the present study.

There are at least two explanations of the discrepancies found between the pharmacodynamics and pharmacokinetics of furosemide. One is put forward by Kaojaren *et al.*,[15] where the time close to the maximally efficient excretion rate of the drug is said to be the determinant of the overall effect. The other explanation is put forward in the present study and in our study in humans,[13] where an acute tolerance development is seen after furosemide administration, the extent of which seems to depend on the time course of delivery of furosemide to the kidneys and on the fluid balance.

The concept of maximally efficient dose or excretion rate proposed by Kaojaren *et al.*[15] is valid if the excretion–response curves after different furosemide administrations are superimposable. Such a condition is probably only obtained by full replacement of fluid throughout the study. Besides, it also seems to be of importance that an immediate replacement is made by an i.v. substitution. Smith *et al.*,[22] in a paper on the furosemide–probenecid interaction in four subjects, found a difference in the excretion–response curve with or without probenecid treatment. The fluid replacement in this study was made by oral substitution. In another paper by Chennavasin *et al.*[5] using more subjects, superimposable excretion–response curves were found for the two treatments. Here, the fluid replacement was made as an i.v. infusion. The differences between the two studies might be explained by the different ways of compensating for the fluid losses.

The concept of acute tolerance development set out in rats in the present study and in humans[13] is more applicable to the disproportionality in effect when the fluid replacement is not complete or when sodium depletion is present, a state more often seen in clinical practice. Under these circumstances, the excretion–response curves are not superimposable.

Thus, the fluid and electrolyte balances are important factors in the feedback inhibition mechanism that is probably triggered when furosemide is given. This mechanism seems to be triggered to different extents depending on the briskness of the furosemide excretion.[13] The nature of this inhibition is not known today. As mentioned earlier, there are many indications in the literature that fluid/electrolyte compensation is the key to different effects of furosemide in different settings. We have in the present study shown that an acute tolerance develops to the diuretic effect of furosemide probably as a consequence of inadequate fluid replacement.

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