Effect of Furosemide on Aminopeptidase Activity in the Rat Kidney and Urine[1]

Many investigators[2] have demonstrated that in the rat by injections of furosemide[3] (4-chloro-N(2-furylmethyl)-5-sulfamoyl-anthranilic acid) the excretion of water, sodium and chloride is markedly increased. Clearance and micropuncture studies indicate that there is an effect of the anthranilic acid derivate on the activity of the reabsorption mechanism in the proximal tubule and in the ascending limb of Henle's loop[4–7]. As has been shown in the rat and dog kidney, furosemide is excreted in the proximal tubule[8,9]. In experiments using this compound in a high dosage, light microscopic investigations revealed tubular alterations in the rat kidney mainly in the outer stripe of medulla[10]. To determine whether these tubular changes after furosemide application occur in response to induced diuresis with water and electrolyte deprivation or to a direct epithelial effect, arylamidase activity in urine was examined in comparison with histochemical staining of aminopeptidase. The masurement and demonstration of these enzymes are accepted to be sensitive methods for detection of renal damage[11–15].

Material and methods. 63 male and 37 female Wistar rats (200–250 g) were used. The animals were housed in metabolic cages with free access to water. Urine was collected twice daily in 12 h samples. Kidneys were removed in ether-anesthesia at various hours after i.p. injection of furosemide (10–100 mg/kg body wt.). Tissue blocks of 2–3 mm thickness were frozen in CO_2 and sectioned at 5 μ in a cryostat. The staining of aminopeptidase was performed by the method of NACHLAS et al.[16] with L-leucyl-β-naphthylamine; the activity of arylamidase in non-dialysed urine was determined with a testkit 'Boehringer' for L-leucyl-p-nitroanilide. The enzyme activity in urine is expressed in milliunits (mU) per 12 or 24 h. HANSON et al.[17] demonstrated that the activity of a particle-bound aminopeptidase (arylamidase) determined by L-leucyl-β-naphthylamine and by L-leucyl-p-nitroanilide differs kinetically from true leucinaminopeptidase. Histochemically there is a group of 3 or 4 aminopeptidases splitting L-leucyl-β-naphthylamine[18,19]. The histochemical reaction is very sensitive (10^{-9} μmoles/h hydrolyzed substrate)[20].

Results. In the rat kidney the aminopeptidase is mainly confined to the cortex. The proximal tubules show a high, the distal tubules only a slight activity (Figure 1, left part). Staining is more intense in the straight portions of proximal tubules. In all parts of the proximal tubule 2 zones in the cell react predominantly: the brush border and the basal part adjacent to the nucleus. In contrast to male rats the enzyme activity in female rats is very high and often obscurs the tubular lumen. The glomeruli and the ascending limbs of Henle's loop exhibit only a sparse enzyme activity. As shown at the right part of Figure 1 (representing histochemical changes induced by an i.p. body injection of 50 mg furosemide/kg body wt.) there is a diffuse diminution of aminopeptidase activity in all parts of the proximal tubules In all slides from kidneys removed 96 h after the same dose of furosemide, the characteristic pattern of the loss of enzyme activity is absent. The enzyme activity of the brush-

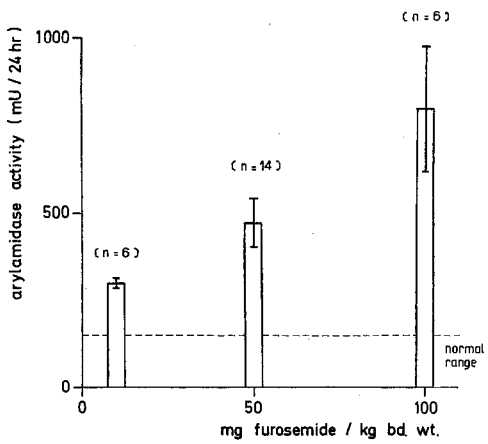

Fig. 2. Arylamidase activity in 24 h urine of male rats after application of several doses of furosemide.

[1] Supported by Deutsche Forschungsgemeinschaft.
[2] Symposion on Lasix; Bad Homburg 1963.
[3] Furosemide = Lasix® (Farbwerke Hoechst A.G., Germany).
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Samples	Arylamidase activity (mU)		Urine volume (ml)		Osmolarity (mosm/L)	
	Control	1 × 50 mg furosemide per kg body wt.	Control	1 × 50 mg furosemide per kg body wt.	Control	1 × 50 mg furosemide per kg body wt.
(a) 12 h (night)	91.80 ± 8.84[a] (n = 27) p = < 0.001	410.11 ± 97.39 (n = 9)	10.33 ± 1.26 (n = 27) n.s.	13.83 ± 1.12 (n = 9)	652.01 ± 52.77 (n = 27) n.s.	524.44 ± 43.31 (n = 9)
(b) 12 h (day)	47.58 ± 3.10 (n = 27) p = < 0.001	127.13 ± 10.22 (n = 9)	3.75 ± 0.85 (n = 27) n.s.	5.53 ± 1.52 (n = 9)	1278.57 ± 80.28 (n = 27) n.s.	1125.33 ± 112.40 (n = 9)

[a] SEM.

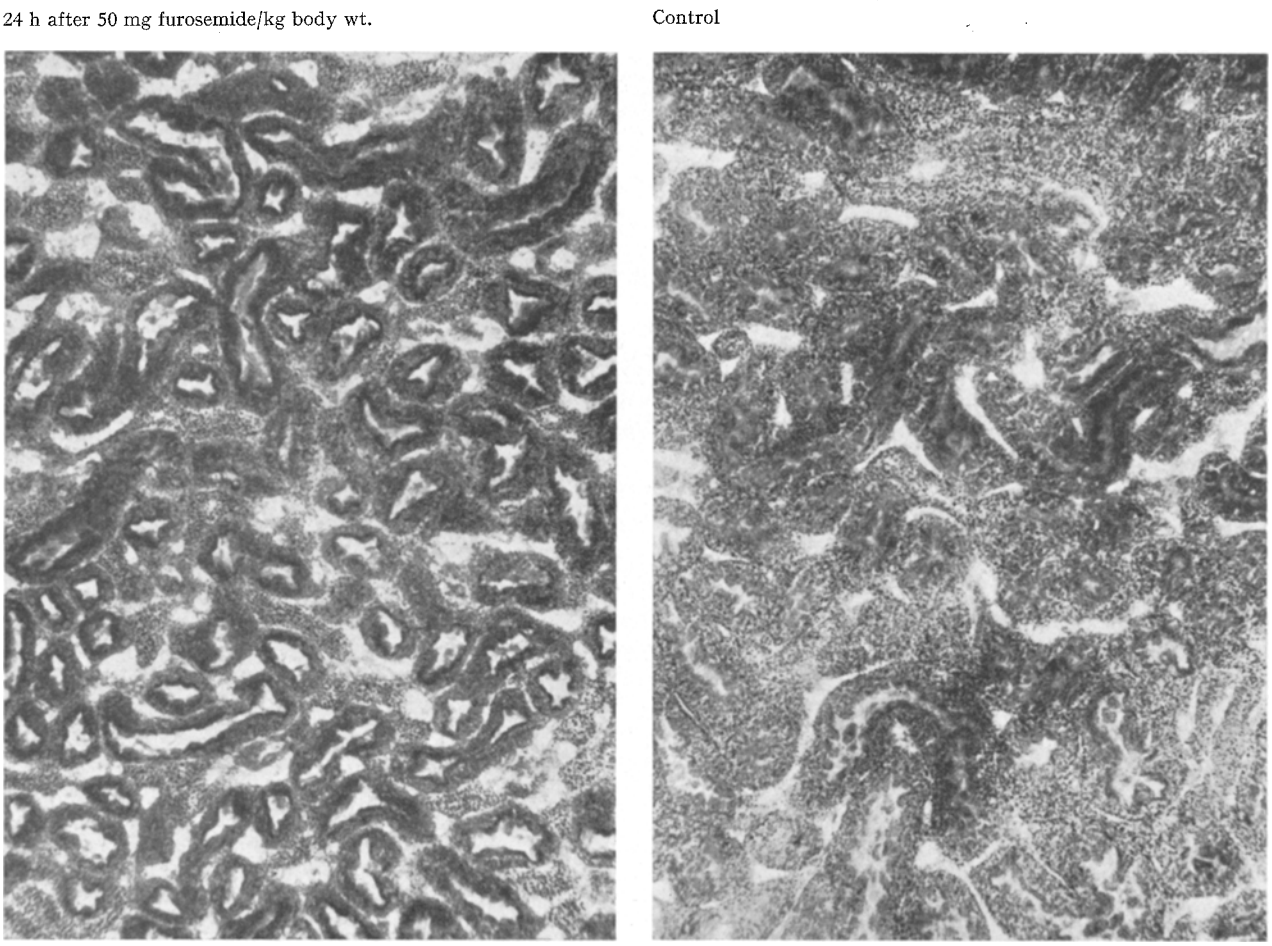

Fig. 1. Male rat kidney (15 min incubation time). Outer stripe of renal medulla. × 160.

border and the basal parts of proximal tubular cells in kidneys removed 1 h after injection of a single dose furosemide (i.e. 10 mg/kg body wt.) is already markedly diminished. Lightmicroscopically no tubular alterations were obtained under these conditions[10].

The Table demonstrates a significant increase of arylamidase activity in the urine of male rats following a single dose of 50 mg furosemide/kg body wt. Figure 2 shows a rising of the arylamidase activity in the urine due to varying doses of furosemide ranging from 10 to 100 mg/kg body wt. It is remarkable that in the kidneys of female rats the histochemical changes are very light and occur only in spots in the outer stripe of the medulla compared to the results in the kidneys of the males. There is a diminution of enzyme activity only in some straight portions of the proximal tubules. No significant elevation of arylamidase activity in urine could be detected, despite repeated massive doses of furosemide (up to 100 mg/kg body wt.). In all cases there is a conspicuous histochemical and biochemical difference between the kidneys of the male and female rats.

Discussion. In contrast to our histological experiences[10], even a single low dose of furosemide is able to change the arylamidase activity in the rat kidney and urine. This fact and the possibility to observe histochemical changes early during the furosemide-induced diuretic phase indicate a direct effect of this compound on the proximal tubular cells. Electron-microscopical studies concerning tubular alterations induced by furosemide support this conclusion[14]. Furthermore the linearity between the dose of furosemide and the quantity of excreted arylamidase refers to augmentation of tubular lesions following enhanced furosemide application. In accordance with these results an increased aminopeptidase excretion in urine of man during furosemide diuresis was reported recently[21]. It is concluded that increased urine arylamidase activity in furosemide treated patients or animals may be caused only by the anthranilic acid derivate and not by tubular damage due to renal diseases[22, 23].

Zusammenfassung. Histochemische und biochemische Untersuchungen des Verhaltens von Aminopeptidasen nach Furosemidgabe zeigen einen Abfall bzw. Anstieg der Enzymaktivitäten in Nierenschnitten und im Urin von männlichen Wistar-Ratten. Die Resultate weisen auf eine direkte, Furosemid-induzierte reversible Epithelschädigung im proximalen Tubulus hin. Unter gleichen Bedingungen sind entsprechende Befunde bei weiblichen Wistar-Ratten nicht zu erheben.

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