Br. J. Pharmacol. (1994), **111**, 173–178

Mechanisms of the hyperkalaemia caused by nafamostat mesilate: effects of its two metabolites on Na^+ and K^+ transport properties in the rabbit cortical collecting duct

[1]Shigeaki Muto, *Masashi Imai & Yasushi Asano

Departments of Nephrology and *Pharmacology, Jichi Medical School, 3311-1 Minamikawachi, Tochigi 329-04 Japan

1 The present experiments were undertaken to determine the mechanism(s) of hyperkalaemia caused by nafamostat mesilate (NM), a serine-protease inhibitor.

2 We investigated the effects of luminal addition of two metabolites of NM, *p*-guanidinobenzoic acid (PGBA) and 6-amidino-2-naphthol (AN), on Na^+ and K^+ transport properties of the collecting duct (CD) cell in the isolated perfused cortical collecting duct (CCD) from rabbit kidneys, because these metabolites, but not NM, were mainly excreted into the urine.

3 Addition of PGBA at 10^{-5} and 10^{-4} M in the lumen resulted in a hyperpolarization of V_A in parallel with increases in transepithelial resistance (R_T) and fractional apical membrane resistance (fR_A). PGBA added to the luminal perfusate at 10^{-5} and 10^{-4} M changed V_A, R_T and fR_A in a dose-dependent manner. These effects were completely inhibited by pretreatment with luminal amiloride (50 μM). PGBA at 10^{-6} M in the lumen had no effect on the electrical parameters.

4 Luminal addition of AN at 10^{-4} M also caused the apical membrane to hyperpolarize in parallel with increases in R_T and fR_A. These effects were also completely inhibited by pretreatment with luminal amiloride (50 μM). AN at 10^{-5} M in the lumen had no effect on the electrical parameters.

5 We conclude that two metabolites of NM, PGBA and AN, act on the apical membrane of the CD cell and inhibit the amiloride-sensitive Na^+ conductance, resulting in an inhibition of K^+ secretion. This direct action of these metabolites, rather than NM, on the CCD might contribute to the NM-induced hyperkalaemia.

Keywords: Nafamostat mesilate; *p*-guanidinobenzoic acid; 6-amidino-2-naphthol; electrophysiology; collecting duct cell; Na^+ conductance

Introduction

Nafamostat mesilate, 6-amidino-2-naphthyl *p*-guanidino-benzoate dimethanesulphonate (NM, Torii Pharmaceutical Co., Japan), is a novel protease-inhibiting agent (Aoyama *et al.*, 1984) used for treatment of pancreatitis (Iwaki *et al.*, 1986) and disseminated intravascular coagulation (DIC) (Yoshikawa *et al.*, 1983; Hitomi *et al.*, 1985; Takahashi *et al.*, 1989). It has also been widely used in haemodialysis as an anticoagulant in patients with bleeding tendencies (Akizawa *et al.*, 1985; Yamazaki *et al.*, 1989; Ohtake *et al.*, 1991).

Recently, it has been reported that continuous intravenous infusion of NM to the patients with pancreatitis or DIC occasionally causes hyperkalaemia due to reduced urinary excretion of K^+ (Okamoto *et al.*, 1990; 1992). However, the exact mechanism(s) underlying this disorder are not fully understood. Very recently, Muto *et al.* (1993) examined the effects of NM on Na^+ and K^+ transport properties in the isolated perfused cortical collecting duct (CCD), which is one of the main sites of K^+ secretion in the kidney (Wright & Giebisch, 1992), and demonstrated the direct action of NM on the CCD. They found that NM mainly acted on the apical membrane of the collecting duct (CD) cell, which plays an important role in Na^+ and K^+ transport in the CCD (Koeppen *et al.*, 1983; Muto *et al.*, 1987a,b; 1988), and that it inhibited the amiloride-sensitive Na^+ conductance. These findings suggest that the direct inhibitory action of NM on the apical membrane Na^+ conductance might contribute, at least in part, to its hyperkalaemic effect. Although the renal handling of NM has not yet been elucidated, it has been reported that NM is metabolized to *p*-guanidinobenzoic acid (PGBA) and 6-amidino-2-naphthol (AN) (Figure 1), which are inactive forms as protease inhibitors (Aoyama *et al.*, 1985; Yang *et al.*, 1990). In addition, these two metabolites, but not NM, are known to be excreted into the urine in rats (Esumi *et al.*, 1984; Shibuya *et al.*, 1984). These findings strongly suggest the possibility that PGBA and/or AN might directly act from the apical membrane of the CD cell and modulate Na^+ and K^+ transport in the CCD. The purpose of the present study was therefore to determine the effects of luminal addition of PGBA and AN on Na^+ and K^+ transport properties in the isolated perfused CCD from rabbit kidneys.

[1] Author for correspondence.

Methods

Isolation and perfusion of tubules

Japanese female white rabbits weighing 1.5 to 2.5 kg were fed a standard laboratory chow and had free access to tap water. They were anaesthetized with intravenous administration of pentobarbitone (35 mg kg^{-1}). Both kidneys were removed and placed in a dish containing a cold intracellular-fluid-like solution of the following composition (mM): KCl 14, K_2HPO_4 44, KH_2PO_4 14, $NaHCO_3$ 9 and sucrose 160.

Segments of the CCD were dissected from the cortex, and transferred to a bath fixed on an inverted microscope (Diaphot: Nikon, Tokyo, Japan). Each tubule was perfused *in vitro* according to the techniques developed by Burg *et al.* (1966) as modified in this laboratory for use of intracellular microelectrodes (Muto *et al.*, 1990; 1991; 1993). Since the details of the technique have been published previously (Muto *et al.*, 1990; 1991; 1993), they will be presented here briefly. Tubules were suspended between two pipettes. The perfusion rate exceeded 20 nl min^{-1} in all tubules. The distal end of the tubule was held in the collecting pipette with unpolymerized Sylgard 184 (Dow Corning, Midland, MI,

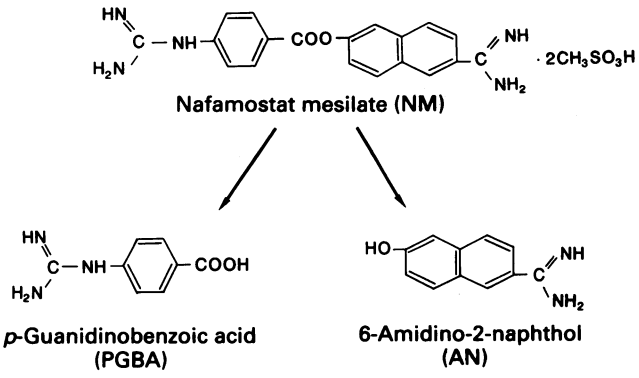

Figure 1 Structural formulae of nafamostat mesilate (NM), *p*-guanidinobenzoic acid (PGBA) and 6-amidino-2-naphthol (AN).

U.S.A.). The tubule was perfused in the bathing chamber of ~100 μl which permits rapid exchange of the bathing solution within 5 s. The bathing solution flowed at 5–15 ml min^{-1} from the reservoirs by gravity through a water jacket to permit the bath temperature to be regulated at 37°C.

Microelectrode studies

The transepithelial and cellular electrical properties of the tubule were measured by techniques used previously in this laboratory and described by Muto *et al.* (1990,1991,1993). The transepithelial voltage (V_T) was measured through the perfusion pipette, which was connected to one channel of a dual electrometer (Duo 773; W-P Instruments, Inc., Sarasota, FLA, U.S.A.) with a 3 M KCl-3% agar bridge and a calomel half-cell electrode. The basolateral membrane voltage (V_B) was measured with 0.5 M KCl-filled microelectrodes, which were fabricated from borosilicate glass capillaries (GD-1.5; 1.5 mm o.d., 1.0 mm i.d.; Narishige Scientific Laboratory, Tokyo, Japan) by using a vertical puller (PE-2, Narishige Scientific Laboratory). Both voltages were referenced to the bath and were recorded on a four-pen chart recorder (R64; Rikadenki, Tokyo, Japan). The electrical potential difference across the apical membrane (V_A) was calculated by the following equation: $V_A = V_T - V_B$.

As previously described (Muto *et al.*, 1990; 1991; 1993), cable analysis was used to calculate the transepithelial resistance (R_T), and the fractional apical membrane resistance (fR_A) [$= R_A/(R_A + R_B)$], where R_A and R_B are the resistances of the apical and basolateral membranes, respectively.

Identification of the CD cell

Electrical identification of the CD cell was performed according to the criteria described previously by Muto *et al.* (1987a, 1990, 1991, 1993). The CD cell has a relatively low fR_A; high V_B; apical Na^+ and K^+ conductances; and basolateral K^+ and Cl^- conductances.

Solutions and materials

The composition of the control bathing and perfusing solution contained (mM): NaCl 10, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1.8, $NaHCO_3$ 25, Na acetate 10, Na_2HPO_4 0.8, NaH_2PO_4 0.2, L-alanine 5 and D-glucose 8.3. This control solution had an osmolality between 285 and 295 mOsm kgH_2O^{-1}, and was equilibrated with 95% O_2/5% O_2 and adjusted to pH 7.4 at 37°C.

Amiloride (Sigma Chemical Co., St. Louis, MO, U.S.A.) was added to the luminal perfusate to achieve a final concentration of 50 μM. PGBA and AN were supplied by Torii & Co., Ltd. (Tokyo, Japan).

Statistics

Experimental values in the text, tables and figures are presented as means ± s.e.mean. Differences between two groups

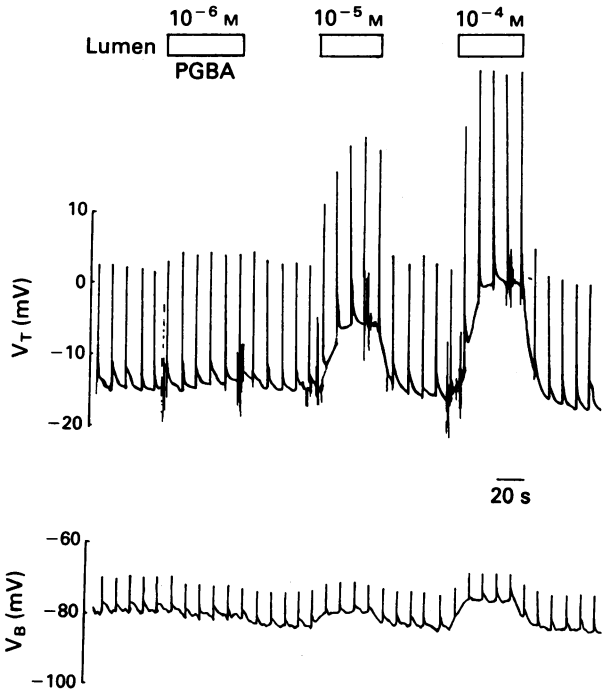

Figure 2 Typical tracings showing effects of *p*-guanidinobenzoic acid (PGBA) in the lumen on V_T and V_B of the collecting duct cell. Voltage spikes are due to 50 nA constant-current pulses at 10 s intervals.

Table 1 Effects of *p*-guanidinobenzoic acid (PGBA) in the lumen on the electrical properties of the collecting duct cell in the rabbit cortical collecting duct

Condition	V_T (mV)	V_B (mV)	V_A (mV)	R_T (Ω·cm²)	fR_A
Control	− 10.2 ± 1.1 (18)	− 77.3 ± 1.6 (18)	67.1 ± 1.4 (18)	106.0 ± 8.1 (15)	0.48 ± 0.03 (15)
PGBA 10^{-4} M (lumen)	− 4.0 ± 0.9* (18)	− 67.5 ± 1.6* (18)	71.6 ± 1.3* (18)	132.8 ± 7.5* (15)	0.65 ± 0.03* (15)
Control	− 10.8 ± 2.0 (9)	− 76.9 ± 1.8 (9)	66.0 ± 1.4 (9)	103.8 ± 9.8 (9)	0.49 ± 0.05 (9)
PGBA 10^{-5} M (lumen)	− 4.8 ± 1.3** (9)	− 72.3 ± 1.4** (9)	67.5 ± 1.7† (9)	113.2 ± 10.0* (9)	0.57 ± 0.05* (9)
Control	− 10.9 ± 2.1 (9)	− 78.2 ± 2.1 (9)	67.3 ± 1.4 (9)	105.3 ± 10.1 (9)	0.49 ± 0.03 (9)
PGBA 10^{-6} M (lumen)	− 10.7 ± 2.0 (9)	− 77.4 ± 2.0 (9)	66.6 ± 1.4 (9)	106.4 ± 10.6 (9)	0.49 ± 0.03 (9)

Values are mean ± s.e.mean; no. of experiments in parentheses. *$P < 0.001$; **$P < 0.005$; †$P < 0.05$ compared with preceding period.

were evaluated with Student's paired t test. Values of $P<0.05$ were considered significant.

Results

Effect of luminal addition of PGBA on electrical properties of the CD cell

To examine whether or not PGBA acts directly on the apical membrane of the CD cell, we added PGBA at 10^{-6}–10^{-4} M to the luminal perfusate and observed the electrical parameters. Figure 2 shows a typical tracing of the changes in V_T and V_B of the CD cell before and after addition of the drug. Table 1 summarizes the effects of PGBA in the lumen on barrier voltages and resistances. PGBA at 10^{-6} M in the lumen had no effect on the barrier voltages or resistances. Significant effects of PGBA on the CCD were observed when the concentrations of PGBA were 10^{-5} and 10^{-4} M. Upon luminal addition of PGBA at 10^{-5} and 10^{-4} M, the lumen-negative V_T was rapidly decreased and the $-V_B$ was slightly decreased, resulting in a significant hyperpolarization of V_A. At that time, both the R_T and the fR_A were significantly increased. However, the changes in V_A, R_T and fR_A upon luminal addition of PGBA were greater at 10^{-4} M than those observed at 10^{-5} M (Figure 3). These results suggest that PGBA at 10^{-5} and 10^{-4} M in the lumen may inhibit the Na^+ conductance at the apical membrane of the CD cell.

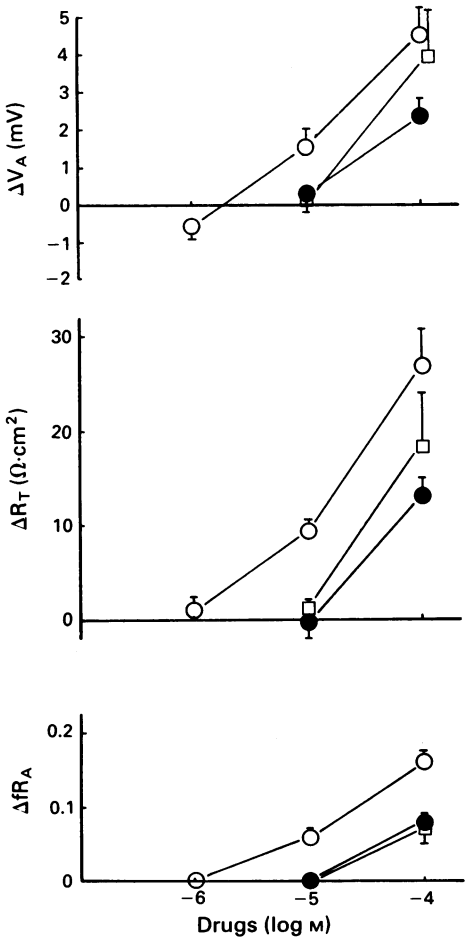

Figure 3 The concentration-response relationships for luminal addition of nafamostat mesilate (NM, □), *p*-guanidinobenzoic acid (○) and 6-amidino-2-naphthol (●) on the changes in V_A, R_T and fR_A. Each point represents the mean of 5–18 separate experiments ± s.e.mean. The data for NM were taken from the paper by Muto *et al.* (1993).

To examine whether this luminal action of PGBA is due to direct inhibition of the apical membrane Na^+ conductance of the CD cell, we added a Na^+ channel inhibitor, amiloride, to the luminal perfusate, and then examined the effect of PGBA on the CD cell. Figure 4 shows a typical tracing of the changes in V_T and V_B of the CD cell before and after addition of luminal PGBA (10^{-4} M) in the presence of luminal amiloride (50 μM). Table 2 summarizes the effects of PGBA in the lumen on the electrical properties of the CD cell in the presence of luminal amiloride. After addition of 50 μM amiloride to the perfusate, the lumen-negative V_T was significantly decreased by 18.1 mV, and the V_B was also significantly depolarized by 10.7 mV, resulting in a hyperpolarization of V_A by 7.3 mV. The R_T and fR_A were also significantly elevated by 26.7 Ω·cm² and 0.12, respectively. However, luminal addition of PGBA in the presence of luminal amiloride produced no further changes in barrier voltages or resistances. These results are consistent with the notion that PGBA inhibits the amiloride-sensitive Na^+ conductance at the apical membrane of the CD cell.

Effect of luminal addition of AN on electrical properties of the CD cell

To examine whether AN also acts on the apical membrane of the CD cell, we added AN at 10^{-5} or 10^{-4} M to the luminal perfusate, and observed the barrier voltages and resistances. Figure 5 shows a typical tracing of the changes in V_T and V_B of the CD cell before and after addition of the drug. Table 3 summarizes the effects of luminal addition of AN on barrier voltages and resistances. AN at 10^{-5} M in the lumen had no effect on the electrical parameters. When AN at 10^{-4} M was added in the lumen, the lumen-negative V_T was significantly decreased by 7.9 mV, and the V_B was also significantly depolarized by 5.7 mV, resulting in a significant hyperpolarization of V_A by 2.2 mV. At that time, both the R_T and the fR_A were significantly increased by 13.4 Ω·cm² and 0.09, respectively. These results suggest that AN in the lumen also

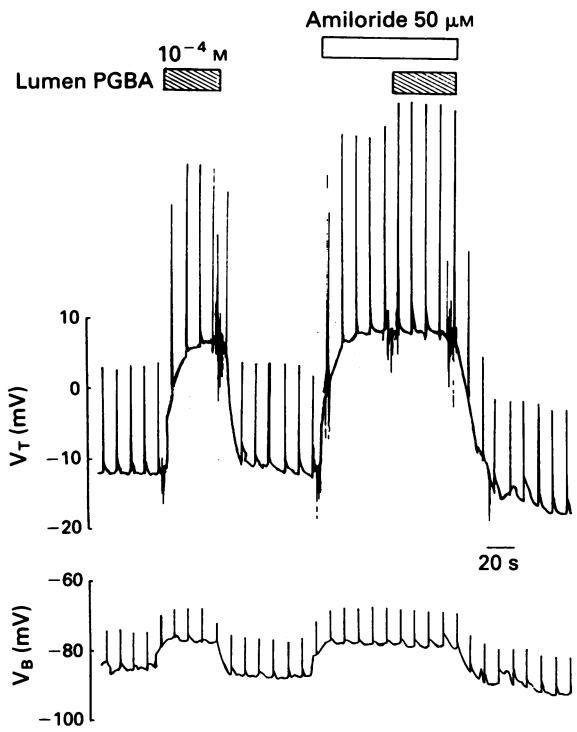

Figure 4 Typical tracings showing effects of *p*-guanidinobenzoic acid (PGBA) in the lumen on V_T and V_B of the collecting duct cell in the presence of luminal amiloride. Voltage spikes are due to 50 nA constant-current pulses at 10 s intervals.

Table 2 Effects of *p*-guanidinobenzoic acid (PGBA) in the lumen on the electrical properties of the collecting duct cell in the presence of luminal amiloride

Condition	V_T (mV)	V_B (mV)	V_A (mV)	R_T (Ω·cm²)	fR_A
Control	− 9.8 ± 1.0 (14)	− 78.9 ± 2.2 (14)	69.1 ± 1.8 (14)	112.0 ± 6.9 (12)	0.46 ± 0.04 (12)
Amiloride 50 μM (lumen)	8.3 ± 0.9* (14)	− 68.2 ± 2.3* (14)	76.4 ± 2.9* (14)	138.7 ± 6.0* (12)	0.58 ± 0.03* (12)
Amiloride 50 μM (lumen) + PGBA 10^{-4} M (lumen)	8.5 ± 0.9 (14)	− 68.3 ± 2.2 (14)	76.8 ± 2.1 (14)	139.1 ± 6.0 (12)	0.58 ± 0.03 (12)

Values are mean ± s.e.mean; no. of experiments in parentheses. *$P < 0.001$ compared with preceding period.

Table 3 Effects of 6-amidino-2-naphthol (AN) in the lumen on the electrical properties of the collecting duct cell in the rabbit cortical collecting duct

Condition	V_T (mV)	V_B (mV)	V_A (mV)	R_T (Ω·cm²)	fR_A
Control	− 7.4 ± 0.8 (16)	− 81.3 ± 2.5 (16)	73.9 ± 2.9 (16)	100.6 ± 6.0 (15)	0.50 ± 0.02 (15)
AN 10^{-4} M (lumen)	0.5 ± 1.4* (16)	− 75.6 ± 3.2* (16)	76.1 ± 2.9* (16)	114.0 ± 6.9* (15)	0.59 ± 0.02* (15)
Control	− 5.9 ± 0.9 (8)	− 80.8 ± 3.8 (8)	74.9 ± 3.7 (8)	119.1 ± 8.6 (7)	0.51 ± 0.04 (7)
AN 10^{-5} M (lumen)	− 5.8 ± 0.9 (8)	− 81.0 ± 3.7 (8)	75.2 ± 3.6 (8)	119.4 ± 9.0 (7)	0.50 ± 0.04 (7)

Values are mean ± s.e.mean; no. of experiments in parentheses. *$P < 0.001$ compared with preceding period.

Table 4 Effects of 6-amidino-2-naphthol (AN) in the lumen on the electrical properties of the collecting duct cell in the presence of luminal amiloride

Condition	V_T (mV)	V_B (mV)	V_A (mV)	R_T (Ω·cm²)	fR_A
Control	− 6.9 ± 2.4 (6)	− 85.6 ± 1.7 (6)	78.7 ± 2.4 (6)	114.8 ± 19.8 (6)	0.50 ± 0.03 (6)
Amiloride 50 μM (lumen)	9.2 ± 1.8* (6)	− 77.1 ± 2.5† (6)	86.4 ± 3.7** (6)	146.1 ± 20.3** (6)	0.60 ± 0.03* (6)
Amiloride 50 μM (lumen) + AN 10^{-4} M (lumen)	9.1 ± 1.7 (6)	− 76.5 ± 2.8 (6)	85.6 ± 3.8 (6)	145.3 ± 17.8 (6)	0.58 ± 0.03 (6)

Values are mean ± s.e.mean; no. of experiments in parentheses. *$P < 0.001$, **$P < 0.005$, †$P < 0.005$ compared with preceding period.

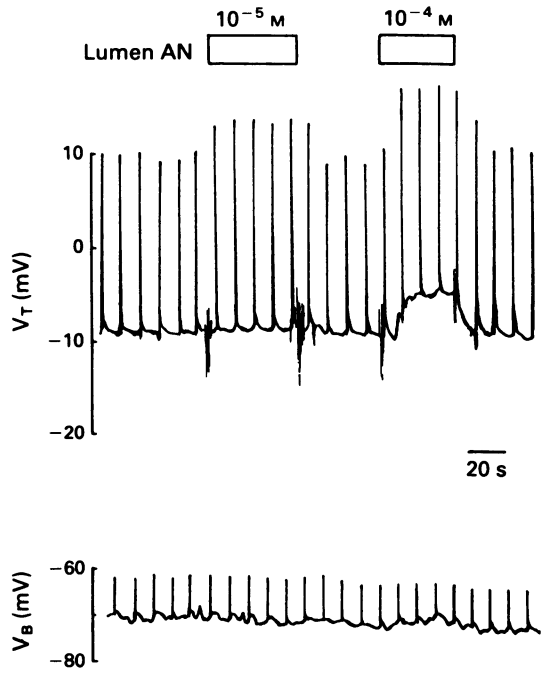

Figure 5 Typical tracings showing effects of 6-amidino-2-naphthol (AN) in the lumen on V_T and V_B of the collecting duct cell. Voltage spikes are due to 50 nA constant-current pulses at 10 s intervals.

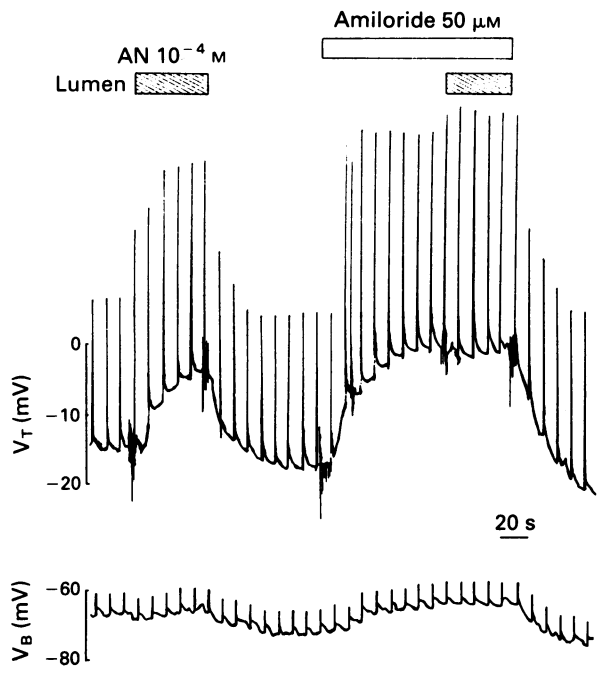

Figure 6 Typical tracings showing effects of 6-amidino-2-naphthol (AN) in the lumen on V_T and V_B of the collecting duct cell in the presence of luminal amiloride. Voltage spikes are due to 50 nA constant-current pulses at 10 s intervals.

inhibits the apical membrane Na^+ conductance of the CD cell.

To examine this possibility, we determined the luminal effects of AN on the barrier voltages and resistances of the CD cell in the presence of luminal amiloride. Figure 6 shows a typical tracing of the changes in V_T and V_B of the CD cell before and after addition of luminal AN (10^{-4} M) in the presence of luminal amiloride (50 μM). Table 4 summarizes the effects of AN in the lumen on the electrical properties of the CD cell in the presence of luminal amiloride. When amiloride was added to the luminal perfusate, the lumen-negative V_T was significantly decreased by 16.1 mV, and the V_B was also significantly depolarized by 8.5 mV, resulting in a significant hyperpolarization of V_A by 7.7 mV. However, luminal addition of AN in the presence of luminal amiloride had no effects on these electrical parameters. Taken together, these results indicate that AN also inhibits the amiloride-sensitive Na^+ conductance at the apical membrane of the CD cell.

Discussion

This study was designed to determine the mechanism(s) of hyperkalaemia caused by NM. The present study focused especially on the luminal action of two metabolites of NM, PGBA and AN, on Na^+ and K^+ transport properties in the CCD *in vitro*, because both metabolites are secreted into the urine and are in contact with the tubular cell apical membrane. We observed that luminal addition of PGBA or AN brings about changes in the electrophysiological behaviour of the CD cell quite similar to those of luminal addition of NM. Thus, the most important findings of our study were that both PGBA and AN act on the apical membrane of the CD cell to inhibit the amiloride-sensitive Na^+ conductance.

The mechanism for Na^+ reabsorption and K^+ secretion in the CCD is well established (Koeppen *et al.*, 1983; O'Neil & Sansom, 1984; Sansom & O'Neil, 1985 and 1986; Muto *et al.*, 1987a,b; Sansom *et al.*, 1989). For Na^+ reabsorption, the first step is passive diffusion of Na^+ from the tubular lumen into the cell via amiloride-sensitive Na^+ conductance across the apical membrane. Na^+ is then actively extruded from the cell across the basolateral membrane via the Na^+-K^+-ATPase pump, thereby maintaining a low intracellular Na^+ activity. On the other hand, the first step in K^+ secretion is active uptake across the basolateral membrane via the Na^+-K^+-ATPase pump, thereby maintaining a high intracellular K^+ activity. K^+ can then diffuse passively across the apical membrane via Ba^{2+}-sensitive K^+ conductance to bring about net K^+ secretion. In the present study, we found that both PGBA and AN acted from the apical membrane of the CD cell and inhibited the Na^+ conductance in the apical membrane. This notion is directly supported by the hyperpolarization of V_A and increases in R_T and fR_A observed after addition of these two drugs in the lumen. Furthermore, pretreatment with luminal amiloride produced no further effects of PGBA or AN in the lumen on the barrier voltages or resistances. Taken together, these results indicate that luminal addition of PGBA or AN results in an inhibition of the amiloride-sensitive Na^+ conductance in the apical membrane. Therefore, the major mechanism of action of both PGBA and AN is to prevent the Na^+ entry into the cell across the apical membrane; the ensuing reduction in the favourable electrical gradient for K^+ secretion from cell to lumen could explain the inhibition of K^+ transport.

Figure 2 shows the comparison of the concentration-response relationships for luminal addition of NM, PGBA and AN on the changes in V_A, R_T and fR_A. It should be noted that luminal application of PGBA at 10^{-5} M caused significant changes in V_A, R_T and fR_A, whereas luminal NM and AN at the same concentration had no significant effects on electrical parameters. These findings strongly suggest that the inhibitory action of PGBA on the apical membranes Na^+ conductance may contribute in a major way to the mechanism of hyperkalaemia caused by NM.

It is also noteworthy that all the electrical parameters returned to the control levels within 30 s after the drugs were eliminated. This would mean that hyperkalaemia induced by NM could be improved simply by interrupting the administration of the drug.

Although we found that PGBA at 10^{-5} and 10^{-4} M, and AN at 10^{-4} M act directly on the apical membrane, the question arises as to whether the concentration of PGBA and AN in the tubular lumen *in vivo* comes in the range of 10^{-5} to 10^{-4} M. At the present time, no clear answer is available on this point. However, when NM was given intravenously to rats at a dose of 1 mg kg^{-1}, the urinary concentration of PGBA and AN reached the level of 10^{-5} and 10^{-6} M respectively, whereas little NM was excreted into the urine (Torii Pharmaceutical Company, personal communication). These findings suggest the possibility that under certain conditions, the concentration of two metabolites of NM in the CCD could reach the level of 10^{-5} to 10^{-4} M, exerting natriuresis and antikaliuresis. Further studies will be required to determine whether PGBA and AN, in fact, cause the natriuresis and antikaliuresis.

In summary, two metabolites of NM, PGBA and AN, act mainly on the apical membrane of the CD cell and inhibit the amiloride-sensitive Na^+ conductance in the apical membrane, resulting in an inhibition of K^+ secretion. PGBA is most potent in this action. This direct action of these two metabolites, rather than NM, could contribute to the NM-induced hyperkalaemia.

References

AKIZAWA, T., SATO, M., KITAOKA, T., KOSHIKAWA, S., ASANO, Y., HIRASAWA, Y., IIDA, K., MIURA, N., NAKAMURA, K., KAZAMA, M. & OTA, K. (1985). The usefulness of the multi-enzyme inhibitor, nafamostat mesilate, in high bleeding risk haemodialysis. *Proc. Eur. Dial. Transplant Assoc.*, **22**, 336–338.

AOYAMA, T., INO, Y., OZEKI, M., ODA, M., SATO, T., KOSHIYAMA, Y., SUZUKI, S. & FUJITA, M. (1984). Pharmacological studies of FUT-175, nafamostat mesilate. I. Inhibition of protease activity in in vivo and in vitro experiments. *Jpn. J. Pharmacol.*, **35**, 203–227.

AOYAMA, T., OKUTOME, T., NAKAYAMA, T., YAEGASHI, T., MATSUI, R., NONOMURA, S., KURUMI, M., SAKURAI, Y. & FUJII, S. (1985). Synthesis and structure-activity study of protease inhibitors. IV. Amidinonaphthols and related acyl derivatives. *Chem. Pharm. Bull.*, **33**, 1458–1471.

BURG, M.B., GRANTHAM, M., ABRAMOV, S. & ORLOFF, J. (1966). Preparation and study of fragments of single rabbit nephrons. *Am. J. Physiol.*, **210**, 1293–1298.

ESUMI, Y., TAKAICHI, M., WASHINO, T., JIN, Y. & KAKURAI, Y. (1984). Pharmacokinetic studies of FUT-175 (Nafamostat mesilate) (6) – blood level profiles, distribution, metabolism and excretion in rats after constant-rate infusion. *Clin. Rep.*, **18**, 4050–4066. (Abs. in English).

HITOMI, Y., IKARI, N. & FUJII, S. (1985). Inhibitory effect of a new synthetic protease inhibitor (FUT-175) on the coagulation system. *Haemostasis*, **15**, 164–168.

IWAKI, M., INO, Y., MOTOYOSHI, A., OZEKI, M., SATO, T., KURUMI, M. & AOYAMA, T. (1986). Pharmacological studies of FUT-175, nafamostat mesilate. V. Effects on the pancreatic enzymes and experimental acute pancreatitis in rats. *Jpn. J. Pharmacol.*, **41**, 155–162.

KOEPPEN, B.M., BIAGI, B.A. & GIEBISCH, G.H. (1983). Intracellular microelectrode characterization of the rabbit cortical collecting duct. *Am. J. Physiol.*, **244**, F35–F47.

MUTO, S., FURUYA, H., TABEI, K. & ASANO, Y. (1991). Site and mechanism of action of epidermal growth factor in rabbit cortical collecting duct. *Am. J. Physiol.*, **260**, F163–F169.

MUTO, S., GIEBISCH, G. & SANSOM, S. (1987a). Effects of adrenalectomy on CCD: evidence for differential response of two cell types. *Am. J. Physiol.*, **253**, F742–F752.

MUTO, S., GIEBISCH, G. & SANSOM, S. (1988). An acute increase of peritubular K stimulates K transport through cell pathways of CCT. *Am. J. Physiol.*, **255**, F104–F114.

MUTO, S., IMAI, M. & ASANO, Y. (1993). Effect of nafamostat mesilate on Na^+ and K^+ transport properties in the rabbit cortical collecting duct. *Br. J. Pharmacol.*, **109**, 673–678.

MUTO, S., SANSOM, S. & GIEBISCH, G. (1987b). Effects of high K diet on electrical properties of cortical collecting duct from adrenalectomized rabbit. *J. Clin. Invest.*, **81**, 376–380.

MUTO, S., YASOSHIMA, K., YOSHITOMI, K., IMAI, M. & ASANO, Y. (1990). Electrophysiological identification of α- and β-intercalated cells and their distribution along the rabbit distal nephron segments. *J. Clin. Invest.*, **86**, 1829–1839.

OHTAKE, Y., HIRASAWA, H., SUGAI, T., ODA, S., SHIGA, H., MATSUDA, K. & KITAMURA, N. (1991). Nafamostat mesilate as anticoagulant in continuous hemofiltration and continuous hemodiafiltration. *Contr. Nephrol.*, **93**, 215–217.

OKAMOTO, T., MARUKAWA, S., TSUDA, S., HAYAMI, H., OZAKI, K., KONO, K. & ISHIDA, H. (1990). Effect of nafamostat mesilate on plasma potassium. *J. Clin. Exp. Med.*, **154**, 777–778. (Abs. in English).

OKAMOTO, T., MARUKAWA, S., HAYAMI, H., OZAKI, K., ISHIDA, H. & KONO, K. (1992). Effect of nafamostat mesilate on renin-aldosterone system. *Masui*, **41**, 329–330. (Abs. in English).

O'NEIL, R.G. & SANSOM, S.C. (1984). Electrophysiological properties of cellular and paracellular conductive pathways of the rabbit cortical collecting duct. *J. Membr. Biol.*, **82**, 281–295.

SANSOM, S.C., AGULIAN, S., MUTO, S., ILLIG, V. & GIEBISCH, G. (1989). K activity of CCD principal cells from normal and DOCA-treated rabbits. *Am. J. Physiol.*, **256**, F136–F142.

SANSOM, S.C. & O'NEIL, R.G. (1985). Mineralocorticoid regulation of apical cell membrane Na^+ and K^+ transport of the cortical collecting duct. *Am. J. Physiol.*, **248**, F858–F868.

SANSOM, S.C. & O'NEIL, R.G. (1986). Effects of mineralocorticoids on transport properties of cortical collecting duct basolateral membrane. *Am. J. Physiol.*, **251**, F743–F757.

SHIBUYA, M., SASAKI, H., KUROTORI, M., IWAMOTO, S. & KURUMI, M. (1984). Pharmacokinetics of FUT-175 (nafamostat mesilate) (4) – metabolism in rats and dogs. *Clin. Rep.*, **18**, 4023–4034. (Abs. in English).

TAKAHASHI, H., TAKIZAWA, S., TATEWAKI, W., NAGAI, K., WADA, K., HANANO, M. & SHIBATA, A. (1989). Nafamostat mesilate (FUT-175) in the treatment of patients with disseminated intravascular coagulation. *Thromb. Haemost.*, **62**, 372.

WRIGHT, F.S. & GIEBISCH, G. (1992). Regulation of potassium excretion. In *The Kidney: Physiology and Pathophysiology*. 2nd ed., ed. Seldin, D.W. & Giebisch, G. pp. 2209–2278. New York: Raven Press.

YAMAZAKI, Z., HIRAISHI, M., KANAI, F., TAKAHAMA, T., IDEZUKI, Y. & INOUE, N. (1989). Pharmacodynamics of FUT-175 anticoagulant in adsorbent plasma perfusion. *Trans. Am. Artif. Intern. Organs*, **35**, 567–569.

YANG, H., HENKIN, J., KIM, K.H. & GREER, J. (1990). Selective inhibition of urokinase by substituted phenylguanidine: quantitative structure-activity relationship analyses. *J. Med. Chem.*, **33**, 2956–2961.

YOSHIKAWA, T., MURAKAMI, M., FURUKAWA, Y., KATO, H., TAKEMURA, S. & KONDO, M. (1983). Effects of FUT-175, a new synthetic protease inhibitor on endotoxin-induced disseminated intravascular coagulation in rats. *Haemostasis*, **13**, 374–378.

(Received August 24, 1993
Revised September 2, 1993
Accepted September 28, 1993)