Upsala J Med Sci 92: 19–35, 1987

Some Effects of Metolazone on Electrolyte Transport

Bo Odlind,[1] Björn Beermann,[2] Björn Lindström,[3] and Örjan Eriksson[3]

[1]*Departments of Medicine, University Hospital, Uppsala,* [2]*S:t Eriks Hospital, Stockholm and* [3]*Department of Drugs, National Board of Health and Welfare, Uppsala, Sweden*

ABSTRACT

Metolazone action was studied 1) in vitro on isolated operculum of Fundulus heteroclitus (active chloride transport) using an Ussing chamber (metolazone conc 500 μM) and in vivo 2) using the modified Sperber technique in the hen (metolazone infusion rate 0.75-1.2 μg/kg/min) and 3) in healthy volunteers using clearance techniques (metolazone infusion rate 10 mg/h). 1) Metolazone reduced ($p<0.05$) short circuit current and potential differences with 20% from average control values ($p<0.05$), while direct current resistance was unchanged. This is comparable to thiazide but much lower than loop diuretic effects. 2) True tubular excretion fraction of metolazone before and after novobiocin (2.7 μmol/kg/min coinfusion averaged 14.1 and 4.5 %, resp. ($p<0.01$; $n=8$). Thus metolazone is partly eliminated by renal tubular secretion. However, the diuretic effect (sodium, chloride and potassium excretion) - and clearances of Cr^{51}-EDTA and I^{125}-Na-o-iodohippurate - were symmetrical, i.e. independent of metolazone urinary excretion rate, as previously shown for thiziades. 3) Renal clearance of metolazone in healthy volunteers. (HPLC-method) averaged 173±20 ml/min ($n=8$). Probenecid (1 g iv.) significantly reduced the renal clearance of metolazone to 33±7 ml/min and potassium excretion with maximum 30%, while diuretic and saluretic effects were significantly increased with maximum 30%. Thus, also in humans the diuretic effect of metolazone is not coupled to the urinary excretion rate of the drug, but suggests that its diuretic effect is elicited primarily from the peritubular side of the nephron. Probenecid apparently dissociates sodium from potassium excretion effects of metolazone. This implies a luminal, sodium-independent kaliuretic effect of the drug.

INTRODUCTION.

Metolazone (7 - chloro - 1,2,3,4, tetrahydro - 2 methyl - 4 oxo - 3 - o - tolyl - 6 quinazolinesulphonamide) is a sulphonamide diuretic chemically related to quinethazone. The diuretic and metabolic effects of metolazone seem

largely to be similar to those of the benzothiadiazines. There are, however, a number of reports about striking diuretic effects of metolazone in patients with severely reduced renal function (7, 8, 17, 19, 36) a condition where "ordinary" thiazides are generally held to be ineffective (33). Some authors have therefore classified metolazone as being more related to the loop diuretics than to the thiazides (43). The aim of this study was to futher characterize the effects of metolazone on electrolyte transport, *in vitro*, using fish operculum and toad urinary bladder (11, 18). Studies were also performed *in vivo*,using the modified Sperber technique in the hen (23) and using conventional clearance techniques in healthy volunteers. The main purpose of the latter studies was to compare metolazone urinary excretion - effect relationship with that of loop diuretics and thiazides as obtained in earlier studies (24-30).

MATERIAL AND METHODS.

Electrophysiological study.

The electrical transepithelial potential difference, short circuit current and direct current resistance were studied in opercular epithelium from *Fundulus heteroclitus*. The fish were kept in 2.5 % artificial sea water for at least 4 weeks after delivery. After killing the animal, the operculum was immediately removed. The epithelium was dissected free from the operculum and was mounted across the central hole of a gasket and placed in an Ussing chamber (9, 11). Metolazone stock solution (27.3 mM) was made by dissolving the compound in Ringer solution alkalinized with NaOH (pH 8.9). Addition of drug increased the pH of the bathing solution 0.03 pH units which itself had no effect on the studied parameters.

The same technique was used to study the effect of metolazone on ion transport in isolated urinary bladder epithelium from *Bufo vulgaris*. (10).The toads were kept in tap water for at least 2 weeks after delivery. The water temperature was 8-10^{o}C and the animals were fed manually with pieces of ox liver once a week. The urinary bladder epithelium was dissected free and placed in an Ussing chamber as described above for the opercular epithelium.

The transport of sodium and chloride was determined electrophysiologically by measuring the transepithelial potential difference (PD; mV), the short-circuit current (SCC; $\mu A \times cm^{-2}$), and the DC resistance (R;Ohm x cm^2). The methods used are described in detail earlier (11).

The following spontaneous activity of the epithelia was observed. The killifish opercular epithelia displayed a transepithelial PD of 10.3$\pm$1.8 mV (seawater side negative), a SCC of 49.9$\pm$4.0 $\mu A \times cm^{-2}$, and a transepithelial DC resistance of 217$\pm$44 Ohm x cm^2 (mean $\pm$ SE;n=4). The corresponding values for

isolated toad bladders were 57.8±4.4 mV (serosal side positive), 82.5±8.7 μA x cm^{-2}, and 722±67 Ohm x cm^2 (mean±SE;n=8).

The modified Sperber technique in the hen.

The same experimental technique was used as has been described in detail and used in similar studies earlier (23, 24, 26). The technique enables separate urine collections from the two injection side (Is) and control side (Cs) ureters and the calculation of the true tubular excretion fraction of metolazone ($TTEF_M$) as

$$\frac{\text{Is - Cs excretion of M}}{\text{Amount of M infused}} \times 100 = TTEF_M\ (\%)$$

A $TTEF_M$ value exceeding 5 % proves active tubular secretion of metolazone (23). C^{14} metolazone was infused into a leg vein on the right side of 8 hens at a rate of 0.75 - 1.2 μg/kg/min. After at least 4-5 collection periods of 10 min at steady state, sodium novobiocin, an inhibitor of organic anion transport (24) was coinfused at a rate of 2.7 μmol/kg/min. The renal clearances of Cr^{51} EDTA (C_{EDTA}) and I^{125}-Na-o-iodohippurate (C_{Hipp}) were determined thoughout the experiments.

Clearance studies in healthy volunteers.

Seven healthy men, 24 to 35 years old, volunteered in the study which was approved by the local Ethics Committee. They arrived at the laboratory in the morning after fasting over night and were supplied with indwelling cannulas in the brachical veins of both arms.

At zero time, i.v. bolus doses of inulin (50 mg/kg) and PAH (10 mg/kg) were given and followed by a continous infusion of 28 and 14 mg/min, respectively. An equilibrium period of at least 1 hour was allowed before starting the clearance determinations. Metolazone was given as an i.v. bolus of 10 mg followed by a continuous i.v. infusion of 10 mg/h 1.5 to 2.5 hrs after the start of the inulin-PAH infusion. Probenecid was administered as an i.v. bolus of 1 g in 50 ml saline at 2.5 hrs after start of the infusion of metolazone, which was continued for another 3 hrs. The subjects emptied their urinary bladders before the experiment. Urine was collected in periods of 30 min and blood samples were drawn within 3 min of the beginning and the end of each collection period. From the start of the experiment to the injection of metolazone the subjects drank 200 ml tap water/h. Urine losses of each period after the injection of the diuretic in excess of the oral water replacement was replaced volume for volume by continuous i.v. infusion of saline during the subsequent

period. Food intake and smoking were not allowed during the experiment. Except when voiding, the subjects were supine during the experiment.

Determination of metolazone.

A liquid chromatographic method was set up for the determination of metolazone in plasma and urine.This utilized a 6000A pump, a U6K injector (Waters Assoc, Milford, MA, USA) and a 970 fluoremetric detector (Spectra Physics, Santa Clara, CA, USA) operated at an exitation wavelength of 230 nm and equipped with a 420 nm emission filter. The column (stainless steel, 150 mm x 4,6 mm I.D.) was slurry packed with Lichrosorb RP-18.5 µm particle size (Merck, Darmstadt, GRF) and eluted with 40% acetonitrile in 0,01 M pH 7 phosphate buffer at a rate of 1.2 ml/min.

The sample (0.5 and 0.1 ml were used for plasma and urine respectively) was added to 0.5 ml of saturated sodium hydrogen-carbonate solution and extracted with 5 ml ethylacetate in a screw-capped tube (shake board 10 min). After centrifugation (500g) 4 ml of the organic phase was removed and evaporated with nitrogen gas. The residue was redissolved in 500 µl of the eluent system and usually a 20 µl aliquot was injected into the liquid chromatograph. These volumes could be adjusted to the concentration range analysed.

The calibration graph was constructed by adding known amounts of metolazone to plasma or urine and analyzing them according to the method described. The resulting peak hights were plotted versus the concentration.

The total recovery when extracting metolazone from plasma (urine) averaged 88%. The precision of the method was 4.5 and 7% (n=10) at 400 and 500 ng/ml plasma, respectively.

Chemical determinations.

Concentrations of inulin and PAH were determined according to Schreiner (37) and Brun (6), respectively. In the volunteer study concentrations of sodium, potassium and chloride were determined according to routine methods at the hospital and in the experimental study as previously described (24).

Isotope determinations.

I^{125} and Cr^{51} activities were determined in a two-channel scintillation spectrometer and carbon-14 activities by liquid scintillation counting.

Materials.

Metolazone and C^{14} metolazone (spec. act. 1.45 mCi/mmol) was obtained from Pharmacia AB, Uppsala Sweden. Probenecid (Injection "Benemid") 0,1 g/ml was

delivered by Merck Frost Research Laboratories, Montreal, Canada. PAH (para-amino-hippurate-sodium) and inulin (Inutest) were supplied by Merck Sharp and Dohme, Rahway, NJ, and Laevosan Gesellschaft, Linz, Austria, respectively. Sodium novobiocin was obtained from Merck Sharp and Dohme, Rahway, NJ. Cr^{51}EDTA was bought from Behringwerke AG, Marburg, Germany and I^{125}-Na-o-iodohippurate from KABI Diagnostika AB, Studvik, Sweden.

Statistical analysis.

Results are given as arithmetic mean ± SD. Differences in clearance or excretion values were analysed according to the concept of confidence intervals. P-values < 0.05 were considered as significant. The difference between pre-drug and post-drug values for fish operculum and toad bladder were evaluated by Student's t-test (two-sided test) for paired variates and considered significant at $P < 0.05$ (*).

RESULTS.

Electrophysiological studies.

Metolazone (500 µM) reduced the short circuit current (SCC) and the potential difference (PD) in the isolated epithelium of Fundulus heteroclitus with about 20% ($p < 0.05$), while the direct current resistance (R) was unchanged (Table I). Metolazone (500 µM) excerted only small effects on PD, SCC and R (-14 to +18%; $P > 0.05$) when administered to the serosal and mucosal side, respectively, of toad urinary bladder (Table I).

Table I. Effects of metolazone on the potential difference (PD), short circuit current (SCC) and direct current resistance (R) in isolated opercular epithelia of Fundulus heteroclitus and in isolated toad bladder.The drug (500 µM) was added to the serosal (S) or mucosal (M) side of the epithelium. The electrical parameters were recorded before and one hour after metolazone exposure. Results are given as per cent change from control values. The number of epithelia tested are given within parentheses (x denotes $p<0.05$).

		PD	SCC	R
		% change from control.		
Opercular epithelium	S (4)	-20.6 ± 2.8^{x}	-19.5 ± 4.5^{x}	-0.1 ± 8.7
Urinary bladder	S (4)	-0.4 ± 6.2	-14.2 ± 7.1	+18.0 ± 11.0
Urinary bladder	M (4)	+6.4 ± 4.9	+15.8 ± 5.5	-5.8 ± 8.2

The modified Sperber technique.

At steady state the $TTEF_M$ value of C^{14} metolazone averaged 14.1 ± 4.2 % (calculated as means of 4-5 collection periods of 10 min from each of 8 animals). After a new steady state had been reached during novobiocin coinfusion, this value was significantly ($p < 0.001$) reduced to 4.5 ± 2.0 % (calculated as means from 3-(4) collection periods of 10 min from each of 8 animals). Corresponding results for total (injection side plus control side) urinary excretion was 58.8 ± 6.0% and 37.7 ± 2.9% ($p < 0.001$), of the infused amount, respectively. The urinary excretion rate of chloride, sodium and potassium as well as C_{EDTA} and C_{Hipp} were symmetrical thoughout all experiments. (cf. Fig 1).

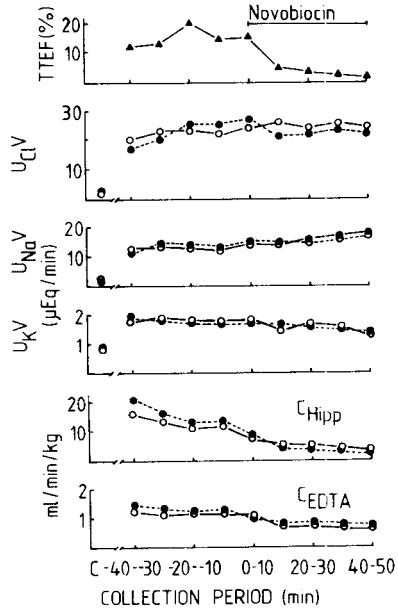

Fig 1.

Typical results from one experiment with the modified Sperber technique. Infusion rate of C^{14} metolazone was 0.9 µg/kg/min. The true tubular excretion fraction of metolazone ($TTEF_M$), sodium, chloride and potassium excretion and the renal clearances of Cr^{51}EDTA and I^{125}-Na-O-iodohippurate from injection and control kidneys, respectively, are shown.

Clearance studies in healthy volunteers.

The plasma levels of metolazone were slowly increasing at the time of injection of probenecid when they averaged 402 ± 64 ng/ml (Fig 2). The renal clearance of metolazone (Fig 2) was almost identical during the two periods before probenecid administration (periods -2 and -1) when it averaged 184 ± 25 and 173 ± 20 ml/min, respectively. Accordingly, period -1 was chosen as control period. The injection of probenecid caused a decrease in renal clearance of metolazone to 33 ± 7 ml/min, which corresponds to 18 ± 2.8 per cent of the

clearance during period -1. The decline in clearance was followed by a continuous rise in plasma levels of metolazone up to 930 ± 204 ng/ml at 3 h after the administration of probenecid (Fig 2).

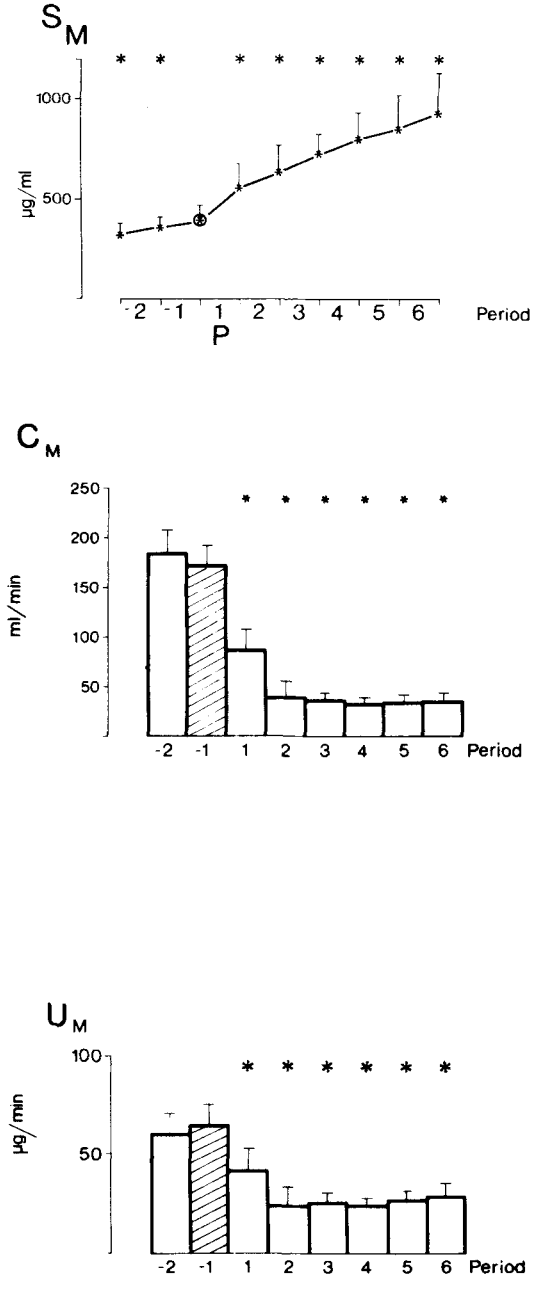

Fig 2.

Plasma levels (S_M), renal clearance (C_M) and urinary excretion (U_M) of metolazone during intravenous infusion of metolazone before and after injection of probenecid (P). * $p<0.05$ in comparison to control period (hatched area).

The urinary flow during the period preceeding the injection of probenecid was 4.1 ± 0.6 ml/min in mean (Fig 3). The injection of probenecid caused a slight but insignificant decrease in diuresis which was followed by a significant mean increase ($p < 0.05$) in excretion of urine, during period 3 and 4 when the diuresis averaged 5.3 ± 1.3 (131.4 ± 32.4 per cent of control) and 4.9 ± 1.6 (120.3 ± 11.0 per cent) ml/min, respectively. The response to the injection of probenecid with regards to time for maximal change differed between individuals. Thus, the maximal individual increase in diuresis averaged 155.9 ± 15.4 per cent of control diuresis ($p < 0.001$).

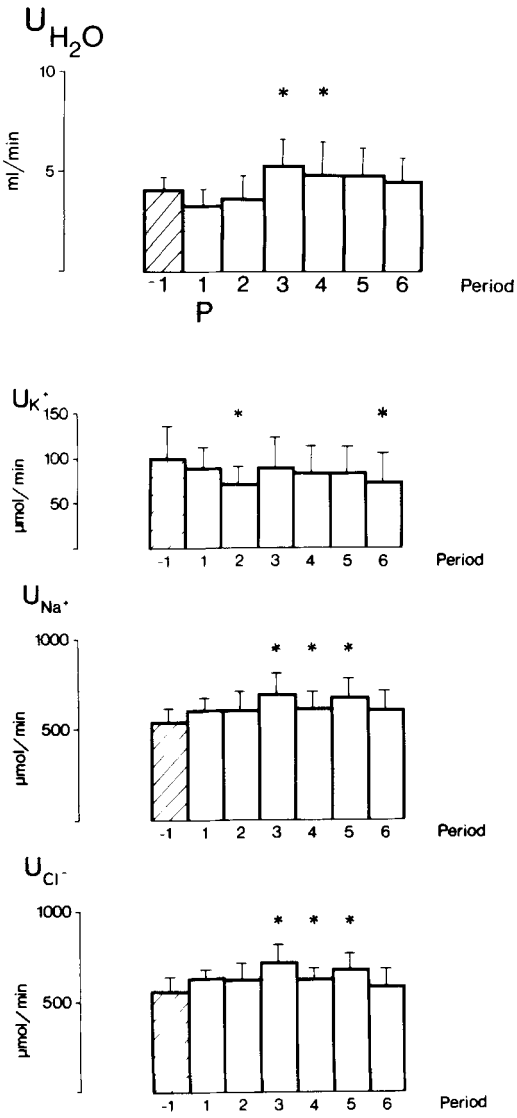

Fig 3 a,b.

Urinary output of water (U_{H_2O}) chloride (U_{Cl^-}), sodium (U_{Na^+}) and potassium (U_{K^+}) during intravenous infusion of metolazone before and after injection of probenecid (P). * $p < 0.05$ in comparison to control period (hatched area).

GFR was 112 ± 14 ml/min during the period before the injection of metolazone. It did not differ from that during the control period when it averaged 109 ± 13 ml/min. Probenecid caused a significant increase in GFR during periods 3 (123 ± 16 ml/min) and 4 (114 ± 9 ml/min). This increase could at least in part explain the increased diuresis during those periods, as corresponding mean fractional excretion of water (EF_{H_2O}) (4.5 ± 1.4 and 4.3 ± 1.3 per cent, respectively) did not differ significantly from that during the control period (3.8 ± 0.5 per cent). However, the maximal individual EF_{H_2O} (6.1 ± 0.6 per cent; period 4.7 ± 1) corresponded to 163.3 ± 25.7 per cent of control ($p < 0.001$). (Fig 4).

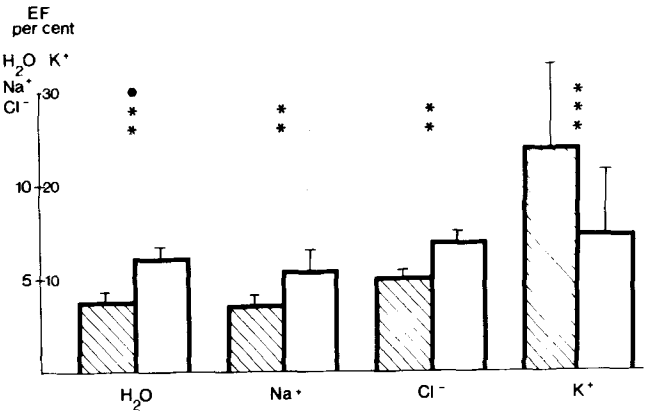

Fig 4.
Maximal change (unfilled area) in fractional excretion (EF) of water, sodium, chloride and potassium during intravenous infusion of metolazone before and after injection of probenecid.
** p<0.01
*** p<0.001 in comparison to control period (hatched area).

Natriuresis averaged 546 ± 73 µmol/min during the control period (Fig 3). After probenecid it was significantly increased ($p < 0.05$) during periods 3-5 with maximal natriuresis averaging 696 ± 117 µmol/min (127.8 ± 13.2 per cent). Again, increased GFR could in part explain the change during periods 3 and 4 as the fractional excretion of sodium (EF_{Na}) did not differ from control value, which amounted to 3.6 ± 0.5 per cent. However, mean EF_{Na} was significantly greater ($p < 0.05$) than control value during period 5 (5.0 ± 1.3 per cent). Also, maximal individual EF_{Na}; 5.4 ± 1.1 per cent was seen during period 5.0 ± 1.1 (150.3 ± 23.4 per cent of control value; $p < 0.01$; Fig 4).

Chloride excretion was significantly higher during periods 3-5 than during the control period when it averaged 560 ± 77 µmol/min. (Fig 3). Maximal chloruresis was 719 ± 103 µmol/min (129.4 ± 16.7 per cent of control) in mean. The fractional excretion of chloride was 5.0 ± 0.4 per cent during the control period. It increased to 5.4 ± 0.5 ($p < 0.05$) and 5.9 ± 0.6 ($p < 0.01$) per cent

during periods 4 and 5, respectively. Mean value for highest individual rise was 6.9 ± 0.6 per cent (period 4.9 ± 1.2); (Fig 5).

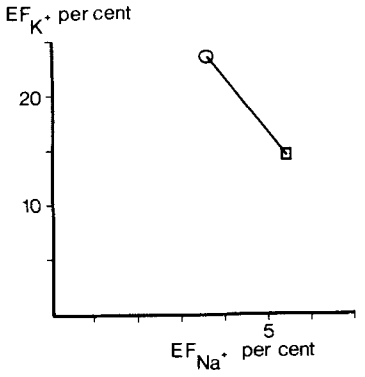

Fig 5.

Dissociation of tubular effects of metolazone on sodium and potassium transport. Fractional excretion of sodium (EF_{Na+}) and potassium (EF_{K+}) before (0) after (¤) probenecid are shown.

The excretion of potassium averaged 99 ± 36 µmol/min during the control period. (Fig 3). The injection of probenecid caused a fall in kaliuresis which was significant ($p < 0.05$) during periods 2 and 6, when it averaged 71 ± 22 (76.1 ± 14.3 per cent) and 77 ± 33 (70.3 ± 28.4 per cent) µmol/min, respectively. The fractional excretion of potassium was 23.8 ± 9.0 per cent during the control period. It decreased to 19.6 ± 7.0 ($p < 0.05$), 17.4 ± 5.1 ($p < 0.01$) and 19.0 ± 6.0 ($p < 0.05$) during periods 1, 2 and 3, respectively. The most pronounced individual decrease was down to 14.6 ± 6.8 per cent ($p < 0.001$) during period 4.4 ± 1.7. (Fig 4).

Renal clearance of PAH decreased from control value of 509 ± 89 to an average of 357 ± 86 ml/min during the six periods following probenecid which corresponds to 70.0 ± 14.8 per cent of control value ($p < 0.01$).

DISCUSSION.

Metolazone (500µM) caused a 20% reduction of the transepithelial potential difference and short circuit current without affecting the DC resistance in isolated opercular epithelia of *Fundulus heteroclitus* (Table I), an epithelium that transports chloride electrogenically (9). This moderate decrease probably reflects unspecific actions of metolazone. "Classical" thiazides e.g. hydrochlorothiazide have no effect on the opercular epithelium while loop diuretics cause a profound inhibition of chloride transport in this epithelium (11).

Moreover, we found no effects of metolazone (500µM) on electrical parameters in the isolated bladder of *Bufo vulgaris* (Table I). This is contrary to the inhibition of SCC and PD reported by Rivecca et al (34)

suggesting an inhibition by metolazone of active sodium transport. The difference could reflect a species difference. Rivecca et al (34) thus used bladders from Bufo marinus, a salt water toad while our observations were made on Bufo vulgaris, a fresh water toad. Results from similar studies with other benzothiazides adds to the complexity. For instance, Pendleton et al (31) found benzoflumethiazide to decrease SCC when added to serosal solution and to slightly increase SCC when added to the mucosal side of the bladder of Bufo marinus. Marumo et al (20) on the other hand found hydrochlorothiazide to cause a partial inhibition of SCC after mucosal addition and no effects on the serosal side of the frog Rana catesbeiana bladder. Schnieders and Ludens (35) observed a 50% decrease of the SCC by 640μM hydrochlorothiazide, added to both sides of the chamber. Thus, it appears that the epithelia studied differ much in their sensitivity to thiazides. Also, it seems that if the effects of different thiazides are to be compared the same epithelium should be used. Interestingly, in a recent study by Stokes et al (40) a thiazide-sensitive electrically neutral transport system has been described in the bladder of the winter flounder. In this system, hydrochlorothiazide and metolazone (but not furosemide) had qualitatively similar actions.

Using the modified Sperber technique it was found that C^{14} metolazone was subject to active tubular secretion in the kidney (Fig 1). The TTEF value of 14% shows a tubular secretion that is smaller than for hydrochlorothiazide (TTEF 59%) in this species (28). Novobiocin coinfusion reduced the steady state TTEF value of metolazone to 1/3 of pre-inhibition values which is evidence for active secretion of metolazone by an organic anion transport system (24). Throughout all experiments, the urinary sodium chloride excretion was symmetrical (Fig 1). Thus, the saluretic effect was not coupled to the tubular secretion of the diuretic. This suggests that the saluretic effect of metolazone is not primarely linked to the urinary excretion of the drug (luminal effect). A predominant action from the blood (peritubular) side of the nephron is therefore proposed, in analogy with previous suggestions for hydrochlorothiazide and chlorothiazide based on similar experiments (28). This then is different from the predominantely luminal effects of loop diuretics, such as furosemide, piretanide or ethacrynic acid (24). Also, the potassium excretion was symmetrical during metolazone infusion (Fig 1) as previously shown for hydrochlorothiazide while chlorothiazide showed a secretion-dependent kaliuresis (28). It has been proposed that this reflects an inhibition of carbonic anhydrase by chlorothiazide in the avian nephron. The lack of a similar effect of metolazone supports this interpretation since metolazone is a poor inhibitor of carbonic anhydrase (12)

In non-hydrated but urine-loss substituted healthy volunteers, metolazone infusion (10 mg bolus and 10 mg/hr) gave clear diuretic but especially saluretic effects with fractional excretion of water and sodium chloride in steady state of 3.5 - 5% (Fig 2 and 3). In previous single dose experiments, maximal fractional sodium excretion of 4 - 7% has been reported following 5 - 10 mg metolazone intravenously (8, 32, 39, 41). Thus, the maximal diuretic and saluretic efficiency of metolazone is much smaller than those of loop diuretics as revieled in similar studies (25, 29, 30). It is of interest that in hydrated subjects metolazone had no (additive) diuretic effect (21, 32, 38, 39).

Use of a steady state experimental technique with continuous infusion of the drug and full substitution of diuretic-induced volume losses has proven successful in previous studies of diuretic excretion-effect relationship in healty volunteers (25, 29, 30). This technique reduces interpretation difficulties that may arise in single dose experiments due to the time course of diuretic excretion (cf. 16) or due to acute tolerance developement.(15)

We found the renal clearance of metolazone in healthy volunteers to average 173 to 184 ml/min in steady state (Fig 4). In view of this and previous reports of a plasma protein binding of metolazone of 95% (42) it is clear that tubular secretion contributes substantially to the renal elimination of the drug in humans. In confirmation of this, probenecid reduced the urinary excretion rate of metolazone and its clearance as much as down to 18% of pre-inhibition values. (Fig 4). In spite of this, probenecid did not reduce the diuretic or saluretic effects of metolazone; on the contrary there was an increase in these effects (Fig 2 and 3). Therefore, it appears that also in humans metolazone excerts its diuretic/saluretic effects primarily from the peritubular (blood) side of the nephron and not from the luminal (urine) side. This is contrary to the case for several loop diuretics (25, 29, 30). A probenecid induced increase in the diuresis/saluresis, similar to our finding for metolazone, has, however, been described by Brater (5) for chlorothiazide, thus adding another thiazide - like property to metolazone.

Interestingly, probenecid decreased metolazone induced absolute and fractional potassium excretion (Fig 3 and 4). The inhibitor therefore dissociated the effects of metolazone on tubular sodium transport from that on potassium transport (cf.Fig 5), which is especially noteworthy since it is generally held that the greater sodium load that appears at the distal tubular sites of sodium-potassium exchange, the greater the output of potassium (cf. 14). Thus, it appears that metolazone could have separate effects on sodium and potassium excretion in healty subjects (Fig 5). Moreover, these results are compatible with a direct luminal kaliuretic effect of metolazone. Metolazone has a

"potassium-sparing" effect, which is different from the well defined potassium-sparing effects of both amiloride and aldosterone-antagonists.(32 However, although an antikaliuretic effect of metolazone has been documented (2), other acute clearance studies in healthy subjects have shown inconsistant effects on potassium excretion (21, 39) or increased potassium excretion (22, 38, 41). In patients with oedematous disorders, metolazone inconsistently cause hyperkaliuria associated with hypokalemia. (19). This might be related to the presence of hyperaldosteronism, since DOCA pretreatment reversed metolazone anti-kaliuresis in healthy subjects. Moreover, in combination - therapy of severe oedematous disoders with loop diuretics and metolazone, excessive potassium excretion and severe hypokalemia coupled to the diuretic effect has been described (4). Therefore, from a clinical point of view metolazone does not seem to offer any advantage over "ordinary" thiazides with regard to potassium losses.

Metolazone infusion caused no changes in GFR as measured by the renal clearance of inulin. This is perhaps not surprising considering that we substituted for urine volume losses for each period and it is in agreement with other reports in hydrated subjects (22, 32, 38-40). Bennett and Porter (2) even found an increased GFR after metolazone while GFR was unchanged after chlorothiazide. This could perhaps be due to different administrations of the two drugs - metolazone was given as a bolus while chlorothiazide was given as a bolus and a sustaining infusion. We found that the GFR increased (significantly during periods 3 and 4) after the probenecid injection. The reason for this is unclear. In our experience from similar studies with other diuretic, probenecid injection has either left GFR unchanged (25) or decreased (29, 30). Renal plasma flow, as determined by the renal clearance of PAH, was as expected significantly reduced by probenecid; an effect of inhibition of PAH secretion.

On the whole, metolazone effects on electrolyte transport seem to be qualitatively similar to those of "classical" thiazides. The fact that metolazone (alone or in combination with a loop diuretic) can be an effective diuretic agent in patients with apparent resistance to diuretics e.g. in renal failure, has been taken as a claim for some unique properties of this drug as compaired to the thiazides. However, some observations with benzothiazides (1, 33) indicate that if the dose is increased, these diuretics may also be effective in similar situations. A clear answer to this question awaits properly designed clinical trials.

Acknowledgments.
The authors are indepted to Mrs Sigrid Sandberg, for excellent technical assistance. We want to thank Pharmacia AB, Uppsala, Sweden for providing the metolazone solutions.

REFERENCES

1. Beermann, B., Odlind, B. and Wibell, L.: Diuretic effect and pharmacokinetics of tizolemide in subjects with normal and decreased renal function. Clin. Nephrol. 19(3):124-131, 1983.
2. Bennett, W. M. and Porter, G.: Comparison of intravenous chlorothiazide and metolazone in normal man. Curr. Ther. Res. 22:326-334, 1977.
3. Bentley, P.J.: Amiloride: A potent inhibitor of sodium transport across the toad bladder. J. Physiol. 195:317-330, 1968.
4. Black, W.D., Shiner, P.T and Roman, J.: Severe electrolyte disturbances associated with metolazone and furosemide. Southern Med. J. 71:380-381, 1978.
5. Brater, C.D.: Increase in diuretic effect of chlorothiazide by probenecid. Clin. Pharmacol Ther. 23 (3):259-265, 1978.
6. Brun, C.: A rapid method for the determination of para-aminohippuric acid in kidney function test. J. Lab. Clin. Med. 37:955-958, 1951.
7. Craswell, P.W., Ezzat, E., Kopstein, J., Varghese, Z. and Moorhead, J.F.: Use of metolazone, a diuretic, in patients with renal disease. Nephron 12:63-73, 1973.
8. Dargie, H.J., Allison, M.E.M., Kennedy, A.C. and Gray, M.J.: Efficacy of metolazone in patients with renal edema. Clin. Nephrol 2(4):157-160, 1974.
9. Degnan, K.J., Karnaky, Jr.K.J. and Zadunaisky, J.A. Active chloride transport in the in vitro opercular skin of a teleost (Fundulus heteroclitus), a gill-like epithelium rich in chloride cells. J. Physiol. 271:155-191, 1977.
10. Eriksson, Ö.: Effects of standard diuretics and orthovanadate on sodium transport across isolated frog skin. Acta Physiol. Scand. 122:249-260, 1984.
11. Eriksson, Ö., Mayer-Gostan, N. and Wistrand, P.J.: The use of isolated fish opercular epithelium as a tissue for studying intrinsic activities of loop diuretics. Acta Physiol. Scand. 125:55-66,1985.
12. Fernandez, P. and Puschett, J.: Proximal tubular actions of metolazone and chlorothiazide. Amer. J. Physiol. 225:4, 954-961, 1973.
13. Frizzell, R.A., Smith, P.L., Vosburgh, E. and Field, M.: Coupled sodium-chloride influx across brush border of flounder intestine. J Membr. Biol. 46:27-39, 1979.

14. Giebisch, G.: Effects of diuretics on renal transport of potassium. In: Martinez - Maldonado M. Editor Methods in Pharmacology. Renal Pharmacology. New York, London, Pleunum Press 4:121-166, 1976.
15. Hammarlund, M.M., Odlind,B. and Paalzow, L.K.: Acute tolerance to furosemide diuresis in humans. Pharmacokinetic-pharmacodynamic modeling. J.Pharmacol Exp. Ther. (In press 1985).
16. Kaojarern, S., Day, B. and Brater, D.C.: The time course of delivery of furosemide into urine is an independent determinant of overall response. Kidney Int. 22:69-74, 1982.
17. Keogh, B., Carmody, M., Malone, D. and O´Dwyer,W.F.: Clinical and biochemical evaluation of a new diuretic agent (metolazone). J. Irish Med. Ass. 64(412) 255-261. 1971.
18. Leaf, A., Andersson, J. and Page, L.B. Active sodium transport by the isolated toad bladder. J. Gen. Physiol. 41:657-668. 1958.
19. Lowenthal, D. and Shear, L.: Use of a new diuretic agent (metolazone) in patients with edema and ascites. Arch Int. Med. 132:38-41, 1973.
20. Marumo, F., Mishina, T. and Shimada, H.: Effects of diazoxide and hydrochlorothiazide on water permeability and sodium transport in the frog bladder. Pharmacology. 24:175-180. 1982
21. Materson, B.J., Hotchkiss, J.L., Barkin, J., Rietberg, B.H., Bailey, K. and Perez-Stable, E.C.: Oral metolazone: Effects on urine composition in water-loaded normal man. Curr. Ther. Research. 14:545-560. 1972.
22. Michelis, M.F., DeRubertis, F., Beck, N.P., McDonald, R. Jr. and Davis, B.B.: Standard oral water load to determine the site of action of diuretics in man. With data on metolazone, a new diuretic. Clin. Pharmacol. Ther. 11:6 821-828. 1970.
23. Odlind, B.: A modified sperber technique for direct estimation of true renal tubular excretion fraction. Acta Physiol. Scand. 103:404-412, 1978.
24. Odlind, B.: Relation between renal tubular secretion and effects of five loop diuretics. J. Pharmacol. Exp. Ther. 211:238-244, 1979.
25. Odlind, B. and Beermann, B.: Renal tubular secretion and effects of furosemide. Clin. Pharmacol. Ther. 27:784-790, 1980.
26. Odlind, B.: Tubular secretion and effects of tienilic acid in the hen. Eur. J. Pharmacol. 72:233-238, 1981.
27. Odlind, B.: Renal tubular secretion and effects of the alkaline diuretics, amiloride, tizolemide (Hoe 740) and MK 447. Naunyn-Schmiedeberg´s Arch. Pharmacol. 137:357-363, 1981.
28. Odlind, B. and Lönnerholm, G.: Renal tubular secretion and effects of chlorothiazide, hydrochlorothiazide and clopamide. Acta Pharmacol. Toxicol. 51:187-197, 1982.

29. Odlind, B., Beermann, B., Selen, G. and Persson, A.E.G.: Renal tubular secretion of piretanide and its effects on electrolyte reabsorption and tubuloglomerular feedback mechanism. J. Pharmacol. Exp. Ther. 225:742-746, 1983.
30. Odlind, B., Beermann, B. and Lindström, B.: Coupling between renal tubular secretion and effects of bumetanide in man. Clin. Pharmacol. Ther. 34(6):805-809, 1983.
31. Pendleton, R.G., Sullivan, L.P., Tucker, J.M. and Stephenson III, T.E.: The effect of bendroflumethiazide on the isolated toad bladder. J. Pharmacol. Exp. Ther. 164:348-361. 1968
32. Puschett,J.B. and Rastegar, A.: Comparative study of the effects of metolazone and other diuretics on potassium excretion. Clin. Pharmacol. Ther. 15:4 397-405, 1973.
33. Reubi, F.C. and Cottier,P.T.: Effects of reduced glomerular filtration rate on responsiveness to chlorothiazide and mercurial diuretics. Circulation. 23:200-210, 1961.
34. Rivecca, J.N., Vogt, J.M. and Greene, J.A.: The effect of metolazone on electrical characteristics of the isolated toad bladder. Fed. Proc. 36:631, 1977.
35. Schnieders, J.R. and Ludens, J.H.: Comparison of effects of standard diuretics and indanone in isolated toad cornea and bladder. Am. J. Physiol. 238:R70-R75. 1980.
36. Schoonees, R., Mostert, J.W., Moore, R.H., Stetson, J.B. and Murphy,G.P.: Evaluation of metolazone. New York State J. Med. March 1:566-569, 1971.
37. Schreiner, G.E.: Determination of inulin by means of resorcinol. Proc. Soc. Exp. Biol. Med. 74:117, 1950.
38. Smiley, J.W., Onesti, G. and Swartz, C.: The acute effects of metolazone on electrolyte and acid excretion in man. Clin. Pharmacol Ther. 13:3 336-342. 1971.
39. Steinmuller, S.R. and Puschett, J.R.: Effects of metolazone in man: comparison with chlorothiazide. Kidney Int. 1:169-181, 1972.
40. Stokes, J.B., Lee, I. and D´Amico, M.: Sodium chloride absorption by the urinary bladder of the winter flounder. A thiazide-sensitive electrically neutral transport system. J. Clin. Invest. 74:7-16. 1984.
41. Suki, W., Dawoud, F., Eknoyan, G. and Martinez-Maldonado, M.: Effects of metolazone on renal function in normal man. J. Pharm. Exp. Ther. 180:1 6-12, 1972.
42. Tilstone, W.J., Dargie, H., Dargie, E.N., Morgan, H.G. and Kennedy, A.C.: Pharmacokinetics of metolazone in normal subjects and in patients with cardiac or renal failure. Clin. Pharm. Ther. 16:2 322-329, 1974.

43. Webb-Peploe, M.M.: New approaches to the treatment of heart failure. In Topics in therapeutics, Vol 5. Royal College of Physicians of London. Editors: Davies DM and Rawlins M.D; Pitman Medical, Publ.; 137-161, 1979.

Send reprint requests to:
Bo Odlind, MD, Ph.D.
Department of Medicine
University Hospital
751 85 Uppsala
Sweden