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Absence of a losartan interaction with renal lithium excretion in the rat

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1 The interaction of losartan, a non-peptide specific AT_1 receptor antagonist with the renal handling of lithium was analysed in conscious normotensive Wistar rats and compared with the known increase in renal tubular lithium reabsorption induced by the non-steroidal anti-inflammatory drug, indomethacin.

2 The rats were treated for five days with losartan (10 mg kg^{-1} day^{-1}, orally), indomethacin (2.5 mg kg^{-1} day^{-1}, intramuscularly) or their solvents. Lithium chloride (16.7 mg kg^{-1}, i.p.) was given as a single dose on the fifth day; renal functions were then measured.

3 Indomethacin, in the absence of any effect on creatinine clearance, increased renal fractional lithium reabsorption and led to an increase in plasma lithium levels.

4 Losartan did not modify renal lithium handling and its plasma level. No change was observed in renal lithium clearance, the quantity of filtered lithium or the fractional reabsorption of the metal. As expected, losartan had no effect on systolic blood pressure in normotensive rats.

5 In conclusion, our results indicate that losartan, when given orally in the rat at a dose of 10 mg kg^{-1} day^{-1} over five days, does not modify renal lithium handling. They suggest that blockade of the angiotensin II receptors does not interfere with renal lithium reabsorption, which occurs mainly at a proximal tubular site.

Keywords: Losartan; lithium renal excretion; indomethacin

Introduction

The prescribing of lithium salts must be very carefully controlled, on account of their narrow therapeutic range. Diminished renal excretion can increase plasma lithium concentrations to toxic levels. For example, non-steroidal anti-inflammatory drugs (NSAIDs) reduce urinary lithium excretion and lead to intoxication in patients receiving lithium therapy (Imbs *et al.*, 1987). Controlled studies in human subjects (Reimann *et al.*, 1983) and animals (Imbs *et al.*, 1980; Barthelmebs *et al.*, 1992) have demonstrated that this interaction is related to NSAID-induced increase in the fractional tubular reabsorption of lithium.

A modification of urinary lithium excretion has been suspected during treatment with angiotensin I converting enzyme (ACE) inhibitors. Several clinical observations of increased plasma lithium concentration have been reported following the introduction of enalapril, captopril or lisinopril in previously well stabilized patients (Douste-Blazy *et al.*, 1986; Pulik & Lida, 1987; Griffin & Hahn, 1991). In a study on Wistar rats, we observed a decrease in the urinary excretion of lithium after a five day treatment with another ACE inhibitor, ramipril (Barthelmebs *et al.*, 1995). The mechanism governing this interaction has not been established.

The aim of the present study was to analyse variations in renal lithium excretion during inhibition of the renin-angiotensin system by losartan, a non-peptide antagonist specific at AT_1 receptors present in the kidneys of rats and men (Timmermans *et al.*, 1991). Urinary lithium excretion was studied in the conscious rat, using a model validated in our laboratory with NSAIDs (Imbs *et al.*, 1980; Barthelmebs *et al.*, 1992). For this reason, indomethacin was included in the study as a reference treatment.

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Methods

Male Wistar rats (Janvier, Le Genest-St-Isle, France), with a mean weight of 258 ± 1 g (mean ± s.e.mean) were randomized into four groups of 21 animals: one group was treated intramuscularly with indomethacin (2.5 mg kg^{-1} day^{-1}), one group was treated by gavage with losartan (10 mg kg^{-1} day^{-1}) and two control groups were treated with the respective solvents.

Experimental protocol

The experimental protocol was similar to that previously described (Imbs *et al.*, 1980). Briefly, the rats were given their treatment between 08 h 00 min and 10 h 00 min for 5 days. Until the morning of the 5th day, they had free access to drinking water and normal sodium standard food (AO4 pellets, Na^+ content = 0.4%, UAR, Villemoisson/Orge, France). On the 3rd day, 3 h after administration of the treatments, systolic blood pressure was measured in conscious animals by tail plethysmography (Physiograph Desk Model DMP 4A, Narco Bio System Inc., Houston, Texas, U.S.A.). The heart rate was calculated from this recording. In the evening of the 4th day, the animals were placed in individual metabolic cages (Iffa Credo, L'Arbresle, France) for a 14 h adaptation period. On the 5th day, renal functions were measured, using a 2 h urine collection. In addition to their daily treatment at T0, the animals were given an i.p. injection of lithium chloride (16.7 mg kg^{-1} at T0) and two s.c. injections of distilled water (16 ml kg^{-1} at T0 and 8 ml kg^{-1} at T0 + 2h). Hydration of the rats allowed sufficient diuresis for a 2 h long urine sampling between T0 + 2h and T0 + 4h. Care was taken to ensure an empty bladder at the beginning and the end of the clearance period. Blood samples for plasma lithium assays were drawn from animals' tails before and after the urine collection period. At the end of the clearance period, blood was drawn by cardiac puncture under light ether anaesthesia in order to measure plasma creatinine, sodium and potassium levels; plasma renin

activity was determined in animals treated by losartan or its solvent. The urinary lithium, creatinine, sodium, potassium, phosphate and uric acid concentrations were also determined. The entire experimental protocol was conducted in a room with controlled temperature (20.5 ± 0.3°C), relative humidity (61.3 ± 2.9%) and a 12h/12h day-night cycle.

Measurements, analytical methods and calculations

Urine volume was recorded. The following measurements were carried out in the plasma and urine: lithium by atomic emission spectrophotometry (Pye Unicam SP9; Philips, Bobigny, France), creatinine by Jaffé's method (Autoanalyseur II, Technicon, Domont, France), sodium and potassium by indirect potentiometry (Beckman Synchron EL-ISE, Gagny, France). The urinary phosphate concentration was determined after complexing with ammonium molybdate (Beckman Clinical System 700) and the urinary uric acid concentration was determined by the phosphotungstic acid reaction (Technicon). Plasma renin activity was assayed by incubation of plasma at 37°C for 30 min and was expressed in ng of angiotensin I(AI) formed per ml of plasma per hour (ng AI ml^{-1} h^{-1}) (kit SBREN II TP6, CIS Bio International, Gif/Yvette, France). The AI was measured by radioimmunoassay using [^{125}I]-AI (NEN, Du Pont de Nemours, les Ulis, France) and a specific antibody provided by Dr J. Nussberger (Lausanne, Switzerland). Renal electrolyte excretions, clearances and fractional reabsorptions were calculated according to the usual formulae. Endogenous creatinine clearance was used as a measure of the glomerular filtration rate.

Drugs

Losartan (DuP 753, Du Pont Merck Pharmaceutical Company) was administered by intragastric route at a dose of 10 mg kg^{-1} day^{-1} in 2 ml distilled water. At this dose, losartan significantly inhibited the pressor response to angiotensin II (AII) throughout the 24 h period (Wong *et al.*, 1990). In a preliminary study conducted under identical animal hydration and lithium injection conditions as used in the present study, we verified that the pressor response to an i.v. injection of 0.3 μg kg^{-1} of AII (Sigma, St Quentin Falavier, France) 4 h after oral administration of 10 mg kg^{-1} of losartan was inhibited by more than two-thirds.

Indomethacin (Merck, Sharp & Dohme-Chibret, Paris, France) was administered by intramuscular injection at the dose of 2.5 mg kg^{-1} day^{-1}, in a volume of 2 ml kg^{-1} in the form of the N-methyl-D-glucamine salt (1.36 mg kg^{-1}, final pH of the solution = 6).

Losartan solvent (2 ml distilled water kg^{-1} day^{-1}) was administered by gastric route.

Indomethacin solvent, a solution of N-methyl-D-glucamine (Fluka, Buchs, Switzerland) at 1.36 mg kg^{-1} in 2 ml of distilled water (pH adjusted to 6), was administered by intramuscular injection.

Statistics

The results are expressed as the mean ± s.e.mean. Statistical differences between groups were identified by Student's *t* test adapted according to Bonferroni for multiple comparisons. The plasma renin activity values were compared using Student's *t* test. The Pearson correlation coefficient *r* was calculated when appropriate. The analyses were performed with BMDP statistical software (BMDP Statistical Software Ltd, Cork, Ireland). A value of $P < 0.05$ was considered to be significant.

Results

The losartan and indomethacin effects were evaluated by comparison with the group treated with the corresponding solvent, administrated via the same route.

Effects of indomethacin

Indomethacin was found to affect renal lithium excretion in a manner similar to previous results from our laboratory (Imbs *et al.*, 1980): in the absence of any modification of endogenous creatinine clearance, indomethacin decreased the urinary excretion and renal lithium clearance by increasing the tubular reabsorption of the metal (Table 1). Indomethacin also decreased the urinary excretions of sodium and potassium without any effect on diuresis (Table 2). The urinary lithium excretion correlated with the renal sodium, potassium and phosphate excretions (Pearson *r* coefficient, $P < 0.001$). The plasma lithium concentration increased (Table 3). Indomethacin had no effect on the systolic blood pressure (115 ± 4 versus 113 ± 2 mmHg in the solvent-treated group) or heart rate (401 ± 6 versus 408 ± 4 beats min^{-1}) measured in conscious rats, on the third day of treatment.

Effects of losartan

A 5 day-treatment with losartan at a dose of 10 mg kg^{-1} day^{-1} did not alter urinary lithium excretion or renal clearance of the metal (Table 1). Fractional lithium reabsorption remained unchanged as did the plasma lithium concentration (Table 3). Losartan treatment did not affect the urinary excretion of uric acid (Table 2) or any of the other parameters measured in this study (creatinine clearance, sodium, potassium and phosphate excretions or plasma parameters) (Tables 1 to 3). On the 5th day of treatment, plasma renin activity was higher in the animals receiving losartan (Table 3).

Table 1 Effects of losartan and indomethacin on creatinine clearance and renal handling of lithium measured after five days of treatment

Treatment	*Indomethacin*	*Indomethacin solvent*	*Losartan*	*Losartan solvent*
Creatinine clearance (μl min^{-1} 100 g^{-1})	402 ± 20	458 ± 17	431 ± 24	424 ± 27
Lithium filtered (nmol min^{-1} 100 g^{-1})	124 ± 9	125 ± 6	126 ± 9	120 ± 9
Lithium clearance (μl min^{-1} 100 g^{-1})	52 ± 3***	120 ± 6	106 ± 5	100 ± 6
Lithium reabsorption (%)	85.91 ± 0.90***	72.17 ± 1.75	73.28 ± 1.19	74.57 ± 1.04
Lithium excretion (nmol min^{-1} 100 g^{-1})	16.8 ± 1.2***	34.5 ± 2.4	32.5 ± 2.2	29.6 ± 1.9

The results are expressed as mean ± s.e.mean and were compared by Student's *t* test adapted by Bonferroni for multiple comparisons. ***$P < 0.001$ compared to corresponding solvent group; $n = 21$ animals in each group.

Losartan had no significant effect on systolic blood pressure (108 ± 3 versus 115 ± 3 mmHg in the solvent-treated group) or heart rate (423 ± 6 versus 403 ± 3 beats min^{-1}) measured in the third day of treatment. Measures were made at the third hour after treatment, i.e. at any time where urine was collected for the clearance study on the 5th day.

Discussion

This study confirms the effects of indomethacin on renal lithium handling, as previously reported in rats under similar experimental conditions (Imbs *et al.*, 1980; Barthelmebs *et al.*, 1992) and in human subjects (Reimann *et al.*, 1983). Plasma lithium concentration increased, due to the increase in tubular reabsorption of the metal, independently of any significant change in the glomerular filtration rate. Fractional lithium reabsorption in solvent-treated rats was within the physiological range (Thomsen, 1990). The experimental conditions therefore appear to offer an appropriate way of examining any possible interaction with renal lithium handling. Losartan did not modify renal lithium excretion under similar experimental conditions. Treatment with the same dose of losartan (10 mg kg^{-1}, orally) was nevertheless sufficient, in our preliminary study, to inhibit, 4 h after the dose, the pressor response to 0.3 μg kg^{-1} of AII by two-thirds. This result is in line with the work by Wong *et al.* (1990): in the pithed rat, the inhibitory effect of the same dose of losartan persisted at 24 h post-dose, with 50% inhibition of the pressor response to 0.1 μg kg^{-1} of AII. As it is likely that the negative feedback of AII on renin release is mediated by AT_1 receptors, we verified that losartan induced an increase in plasma renin activity in our study, as reported previously in WKY rats after a 7-day treatment (Bunkenburg *et al.*,1991). Losartan did not lower blood pressure in normotensive conscious animals as has been previously described (Wong *et al.*, 1990). It did, however, elicit a sustained antihypertensive effect on SHR, with no tolerance developing after repeated daily oral dosing of 10 mg kg^{-1} (Wong *et al.*, 1991).

Five days treatment with losartan did not alter renal lithium excretion in our study in the rat. Losartan did not affect renal lithium clearance following acute administration to patients (Martinez *et al.*, 1993). Lithium reabsorption mainly occurs in the proximal tubule and can be considered to be a valid indicator of sodium reabsorption in this tubular segment (Leyssac, 1990; Koomans & Dorhout Mees, 1990). Although more distal parts of the nephron have been shown to be permeable to lithium, their contribution to overall lithium reabsorption is small when sodium intake is normal (Greger, 1990; Koomans & Dorhout Mees, 1990). The strong correlation we found between changes in lithium and phosphate excretion after indomethacin treatment confirms the proximal tubular site for lithium reabsorption since phosphate reabsorption occurs mainly in the proximal tubule (Knox *et al.*, 1973).

Table 2 Effects of losartan and indomethacin on the other urinary parameters measured after five days of treatment

Treatment	*Indomethacin*	*Indomethacin solvent*	*Losartan*	*Losartan solvent*
Sodium filtered (μmol min^{-1} 100 g^{-1})	57 ± 3	65 ± 2	61 ± 3	60 ± 4
Sodium reabsorption (%)	99.89 ± 0.01***	99.69 ± 0.04§	99.73 ± 0.04	99.80 ± 0.02
Sodium excretion (μmol min^{-1} 100 g^{-1})	0.06 ± 0.01***	0.20 ± 0.02§§	0.16 ± 0.02	0.12 ± 0.01
Potassium excretion (μmol min^{-1} 100 g^{-1})	0.32 ± 0.02***	0.46 ± 0.03	0.48 ± 0.03	0.41 ± 0.03
Phosphate excretion (nmol min^{-1} 100 g^{-1})	25 ± 4(*)	55 ± 11	55 ± 12	41 ± 9
Uric acid excretion (nmol min^{-1} 100 g^{-1})	6.1 ± 0.4	7.1 ± 0.3	6.6 ± 0.4	6.3 ± 0.5
Diuresis (μl min^{-1} 100 g^{-1})	6.09 ± 0.55	7.41 ± 0.56	7.75 ± 0.74	6.72 ± 0.79

The results are expressed as mean ± s.e.mean and were compared by Student's *t* test adapted by Bonferroni for multiple comparisons. (*) $P<0.10$, ***$P<0.001$ compared to corresponding solvent group; §$P<0.05$, §§$P<0.01$ indomethacin solvent versus losartan solvent; $n=21$ animals in each group.

Table 3 Effects of losartan and indomethacin on the plasma parameters

Treatment	*Indomethacin*	*Indomethacin solvent*	*Losartan*	*Losartan solvent*
Plasma creatinine (μM)	47.8 ± 1.1	44.5 ± 1.2	45.6 ± 1.0	45.0 ± 1.0
Plasma sodium (mM)	147.6 ± 1.7	148.6 ± 1.8	147.2 ± 1.8	147.0 ± 1.2
Plasma potassium (mM)	4.55 ± 0.20	4.49 ± 0.23	4.71 ± 0.24	4.30 ± 0.12
Plasma lithium (mM)	0.322 ± 0.010*	0.287 ± 0.009	0.301 ± 0.010	0.296 ± 0.008
Plasma renin activity (ng AI ml^{-1} h^{-1})	ND	ND	212 ± 18***	61 ± 6

The results are expressed as mean ± s.e.mean and were compared by Student's *t* test adapted by Bonferroni for multiple comparisons or by Student's *t* test, *$P<0.05$, ***$P<0.001$ compared to corresponding solvent group; $n=21$ anmals in each group; ND = not determined.

Lithium can ride on Na^+H^+-antiporter where sodium can be exchanged for lithium. However, it is doubtful whether lithium is extruded from the proximal epithelial cells via the Na^+K^+-ATPase, since this pump has a low affinity for lithium. Proximal lithium reabsorption may therefore occur mainly via the paracellular route (Greger, 1990). This is probably why losartan, which decreases the proximal tubular sodium reabsorption controlled by AII (Cogan *et al.*, 1991), increased sodium, but not lithium clearance after acute administration in healthy volunteers (Martinez *et al.*, 1993). In our study in rats, after a 5-day treatment with losartan, we observed no natriuretic effect. It is possible that any inhibition of proximal tubular sodium reabsorption by losartan was masked by the adaptation of the more distal tubular functions to an increased sodium load.

The present results with losartan suggest that inhibition of the renin-angiotensin system did not directly lead to an interaction with renal lithium handling. Our study however was done in normotensive animals. Whether a similar effect would be observed in hypertensive patients in whom the proximal tubular sodium reabsorption can be different needs further investigation.

A rise in plasma lithium level has been previously reported with several ACE inhibitors when given as antihypertensive therapy in manic depressive patients (Douste-Blazy *et al.*, 1986; Pulik & Lida, 1987; Griffin & Hahn, 1991). In Wistar rats, ramipril increased the plasma lithium concentration by decreasing its renal excretion when measured on the fifth day of the treatment with the ACE inhibitor (Barthelmebs *et al.*, 1995). The experimental protocol was the same as that used in the present study with losartan. Unlike losartan, ramipril induced a slight decrease in the systolic blood pressure. The interaction between ACE inhibitors and renal lithium handling could therefore be linked to changes in renal functions following the decrease in mean blood pressure. In addition, ACE inhibitors potentiate the renal effects of bradykinin, vasodilatation and natriuresis (Vanhoutte *et al.*, 1993). Salt and water depletion could then also be responsible for an increase in lithium reabsorption (Atherton *et al.*, 1990).

In conclusion, a 5-day treatment with losartan, the nonpeptide antagonist of the AT_1 receptors, failed to modify renal lithium handling in normotensive rats. Our data suggest that inhibition of the renin-angiotensin system does not interfere directly with renal lithium reabsorption. The increase in the plasma lithium level which had been reported with ACE inhibitors in human subjects may be linked to another effect like bradykinin potentiation and/or to an effect which only becomes apparent after inhibition of the renin-angiotensin system in a hypertensive state.

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