Effects of a new pteridine derivative on urinary sodium, potassium and magnesium excretion in conscious saline-loaded rats

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1 Two recently synthesized pteridine derivatives (RPH 3036; RPH 3038) were tested in conscious saline-loaded rats and showed natriuretic and antimagnesiuretic properties but hardly reduced potassium excretion.

2 In the same model a dose-response curve was performed for RPH 3036. ED_{50} and E_{max} values were calculated for the natriuretic ($ED_{50} = 13.4 \mu\text{mol kg}^{-1}$; $E_{max} = 1.08 \text{ mmol kg}^{-1}$) and antimagnesiuretic ($ED_{50} = 11.3 \mu\text{mol kg}^{-1}$; $E_{max} = -0.099 \text{ mmol kg}^{-1}$) properties of RPH 3036. There were no significant changes of potassium and calcium excretion.

3 After a single dose of RPH 3036 ($100 \mu\text{mol kg}^{-1}$) the time course of electrolyte excretion was analysed over 6 h. RPH 3036 did not show any significant effects on renal potassium and calcium excretion whereas a pronounced decrease ($P < 0.01$) in renal magnesium excretion was evident during the 6 h. A moderate increase of sodium excretion was observed only after 3, 5 and 6 h.

4 A selective reduction of magnesium secretion in the late distal tubule and collecting duct was proposed as a possible mechanism of action of RPH 3036. This would explain the fast onset of action as well as the lack of antikaliuretic and anticalciuretic effects. The high selectivity of RPH 3036 makes it potentially valuable for the future investigation of renal magnesium transport.

Keywords: Magnesium-sparing diuretics; potassium-sparing diuretics; pteridine derivatives; triamterene derivatives

Introduction

Increasing attention has been drawn over recent years to diuretic-induced magnesium loss and its consequences (Dyckner & Wester, 1984; Learey & Reyes, 1984). Therapeutic strategies to avoid this magnesium loss primarily consist of the use of potassium-sparing diuretics (Dyckner *et al.*, 1986; Widman *et al.*, 1988; 1990) since there is growing evidence for an antimagnesiuretic effect of these substances (Lim & Jacob, 1978; Devane & Ryan, 1981; 1983).

Triamterene, for example, exerted magnesium-sparing properties in laboratory animals (Mutschler *et al.*, 1981) as well as in man (Hänze & Seyberth, 1967). The lack of antimagnesiuretic activity of triamterene in other studies (van Meyel *et al.*, 1990) was probably due to a different study design. Low water solubility and a high biotransformation rate of triamterene might cause adverse side-effects. Therefore, derivatives with better physico-chemical and pharmacokinetic properties were synthesized several years ago. Some of these derivatives not only showed marked antikaliuretic activity in conscious saline-loaded rats but also exerted pronounced magnesium retaining effects after acute administration (Prierer *et al.*, 1985; Ullrich *et al.*, 1990). The magnesium-sparing effect of these triamterene derivatives was more pronounced than that seen after administration of triamterene itself. These derivatives, however, were potent antikaliuretics like all other diuretic agents with antimagnesiuretic activity.

Recently three new pteridine derivatives (Figure 1) were synthesized and the aim of this study was to test 2,4,7-triamino-6-(*N,N*-dimethylaminomethyl)pteridine (RPH 2997), 2,4,7-triamino-6-[2-(*N,N*-dimethylamino)ethyl]pteridine (RPH

3036), and 2,4,7-triamino-6-[3-(*N,N*-dimethylamino) propyl] pteridine (RPH 3038) for their natriuretic, antikaliuretic, and antimagnesiuretic effects in conscious saline-loaded rats. As will be shown later, RPH 3036 and RPH 3038 are the first magnesium-sparing diuretic compounds that are almost devoid of antikaliuretic activity.

Figure 1 Chemical structures of triamterene (1), RPH 2997 (2), RPH 3036 (3), and RPH 3038 (4).

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Methods

Pharmacodynamic experiments

Male Wistar rats weighing 140–210 g were used. Food was withdrawn 18 h before the experiment but tap water was available *ad libitum*. The rats were randomly divided into groups of 5 to 10 animals as indicated in the text. In every experiment the diuretic-treated groups were compared to a control group receiving solvent only. All rats were given by gavage 20 ml kg⁻¹ body weight of a 0.9% NaCl solution. Then the test compound (dissolved in 1 ml kg⁻¹) was injected under light ether anaesthesia in one of the caudal veins. The animals were placed in individual metabolism cages without food and water. All experiments were started at 08 h 00 min. Depending on the experiment urine was collected for 2.5 h and 6 h or for 2, 3, 4, 5, and 6 h, respectively.

Analytical methods

To determine its volume the urine was collected in graduated (± 0.1 ml) centrifuge tubes. Sodium and potassium concentrations were analysed by flame emission spectrophotometry and calcium and magnesium concentrations were determined by atomic absorption spectrophotometry with an Elektrolytautomat FL 6 (Zeiss, Oberkochen, Germany) in each case.

Data analysis

Data are expressed as means $\pm$ s.e.mean. Significance of differences between means was assessed by the Kruskal-Wallis test for the dose-response experiments and by a two-sided U-test according to Wilcoxon, Mann, Whitney for the rest of the experiments.

The fig.P computer programme (Biosoft, Cambridge, U.K.) was used to fit sigmoid curves to the data points of dose-response experiments and to calculate ED₅₀ and E_{max} values. The calculations were based on the following equation:

$$E = E_0 + E_{\max} \frac{D^P}{D^P + ED_{50}^P}$$

where E₀ is the basal response without any drug, D the dose administered, E the response to the dose D, E_{max} the change of E₀, which is induced by the maximally effective dose, ED₅₀ the median of the effective dose and where P is the exponential factor which determines the shape of the curve.

Drugs

Triamterene was a gift from Röhm Pharma GmbH (Weierstadt, Germany). RPH 2997 was used as the HCl salt. All substances were dissolved or suspended in a solution of 20% polyethylene glycol (PEG) 400 and 80% water.

Syntheses

RPH 2997, RPH 3036 and RPH 3038 are not yet described in the literature and were prepared as follows.

2,4,7-Triamino-6-(N,N-dimethylamino)methylpteridine (RPH 2997): 2,4,7-Triamino-6-methylpteridine (1) was synthesized according to Pachter (1963). (1) was converted to 2,4,7-triamino-6-bromomethylpteridine (2) by photobromination. Nucleophilic substitution of (2) with diethylamine resulted in RPH 2997.

2,4,7-Triamino-6-(2-N,N-dimethylaminoethyl)pteridine (RPH 3036): Nucleophilic substitution of 3-chloropropionaldehyde-diethylacetal (Aldrich, Steinheim, Germany) with dimethylamine gave 3-dimethylaminopropionaldehyde-diethylacetal (1). Using (1) and tetraaminopyrimidine (Aldrich, Steinheim, Germany) RPH 3036 was prepared by the same procedure as described by Pachter (1963) except that hydrochloric acid

was used instead of acetic acid as the acetal had to be cleaved. m.p. 286°C (decomp.)

2,4,7-Triamino-6-[3-(N,N-dimethylamino)propyl]pteridine (RPH 3038): RPH 3038 was synthesized in analogy to RPH 3036 starting with the nucleophilic substitution of 2-(3-chloropropyl)-1,3-dioxolane (Fluka, Neu-Ulm, Germany) with dimethylamine. m.p. 226°C (decomp.)

All compounds were characterized by ¹H nuclear magnetic resonance (n.m.r.) and ¹³C n.m.r. measurements as well as by elemental analyses (data not given).

Results

A comparison between the effects of triamterene and the three recently synthesized pteridine derivatives at a dose of 25 μ mol kg⁻¹ ($n = 10$) is shown in Figure 2. During the first collection period triamterene exerted highly significant diuretic ($P < 0.01$), natriuretic ($P < 0.001$) and antikaliuretic ($P < 0.01$) effects whereas RPH 2997 only reduced potassium excretion ($P < 0.01$) without any significant influence on the other parameters. In contrast to triamterene and RPH 2997, both RPH 3036 and RPH 3038 almost selectively exhibited magnesium-retaining properties ($P < 0.01$). Only after administration of RPH 3036 was a minor natriuretic activity ($P < 0.05$) seen. The effects observed after 6 h were hardly different (data not shown): The increase in magnesium excretion ($P < 0.05$), initially induced by triamterene, was not evident after 6 h. The natriuretic effect of RPH 3036 was attenuated after the longer collection period.

To obtain more information about the selectively anti-magnesiuretic compounds, we chose RPH 3036 to perform a dose-response curve ($n = 6$; Figure 3). Due to significant changes of sodium ($P < 0.1$) and magnesium ($P < 0.01$) excretion ED₅₀ and E_{max} values were calculated for natriuretic and antimagnesiuretic effects. The ED₅₀ values for both natriuresis (ED₅₀ = 13.4 μ mol kg⁻¹) and antimagnesiuresis (ED₅₀ = 11.2 μ mol kg⁻¹) were almost the same. The maximal increase in sodium excretion (E_{max}) was calculated to be only 1.08 mmol kg⁻¹ and the maximal decrease in magnesium excretion was

Figure 2 Effects of solvent (□), triamterene (■), RPH 2997 (▨), RPH 3036 (▧), and RPH 3038 (▩) (all 25 μ mol kg⁻¹) on urinary volume, sodium, potassium, and magnesium excretion in the conscious saline-loaded rat. Urine was collected over a 2.5 h period. Each column is the mean of $n = 10$ rats/group; s.e.mean shown by vertical bars. * $P < 0.05$ vs. control; ** $P < 0.01$ vs. control; *** $P < 0.001$ vs. control.

Figure 3 Effects of different doses of RPH 3036 on urinary sodium, potassium, calcium, and magnesium excretion in the conscious saline-loaded rat. Urine was collected over a 2.5 h period. Each point is the mean of $n = 6$ rats/group; s.e.mean shown by vertical bars. A sigmoid curve was fitted to the data of sodium and magnesium excretion by the computer programme fig.P (Biosoft, Cambridge, U.K.). The ED_{50} values of natriuretic and antimagnesiuretic effects were $13.4 \mu\text{mol kg}^{-1}$ and $11.2 \mu\text{mol kg}^{-1}$, respectively.

calculated to be $-0.099 \text{ mmol kg}^{-1}$. There were no significant changes in potassium and calcium excretion.

In the last experiment ($n = 5$) the time course of effects on urinary excretion of water, sodium, potassium, calcium, and magnesium was investigated with a high dose ($100 \mu\text{mol kg}^{-1}$) of RPH 3036 (Figure 4). Sodium excretion was increased after 3 h ($P < 0.05$) and after 5 and 6 h ($P < 0.01$). A pronounced reduction in magnesium excretion ($P < 0.01$) was evident the whole time but no significant potassium-sparing effects were seen. Calcium excretion did not show any significant changes but tended to be slightly higher than the corresponding control values.

Discussion

The results of this study provide evidence that RPH 3036 and RPH 3038 are the first diuretic compounds with prominent antimagnesiuretic but no antikaliuretic or anticalciuretic effects.

In Figure 5 the different potencies, expressed as $-\log ED_{50}$ (mol kg^{-1}), of RPH 3036 were compared to the corresponding values of some other closely related pteridine derivatives such as triamterene, 2,4,7-triamino-6-[4-(3-diamethylamino-2-hydroxypropoxy)phenyl]pteridine (H3), 2,4,7-triamino-6-[4-(3-dimethylaminopropoxy)phenyl]pteridine (B3), ethylamide

Figure 4 Time-dependent effects of RPH 3036 ($100 \mu\text{mol kg}^{-1}$; filled symbols) in conscious saline-loaded rats on urinary volume and electrolyte excretion compared to the effects of solvent (open symbols). Each point is the mean of $n = 5$ rats/group; s.e.mean shown by vertical bars. * $P < 0.05$ vs. control; ** $P < 0.01$ vs. control.

Figure 5 Natriuretic (open columns), antidiuretic (solid columns), and antimagnesiuretic (cross-hatched) properties of triamterene, H3, B3, A2, S3, and RPH 3036 expressed as $-\log ED_{50}$ (mol kg⁻¹) values.

of 2,4,7-triamino-6-[4-(4-carboxybutoxy)phenyl]pteridine (A2), and 2,4,7-triamino-6-[4-(4-carboxybutoxy)phenyl]pteridine (S3) (data taken from Priewer *et al.*, 1985; 1987).

All compounds shared about the same natriuretic potency whereas antidiuretic and antimagnesiuretic potencies varied to a great extent. Although among the previous triamterene derivatives some exerted antidiuretic effects without any influence on magnesium excretion (e.g. B3) none could reduce magnesium excretion without affecting urinary output of potassium. The first compound with this unique profile was RPH 3036 with an antimagnesiuretic potency of 11.2 $\mu\text{mol kg}^{-1}$ but with almost no effect on potassium excretion up to a dose of 100 $\mu\text{mol kg}^{-1}$.

To explain antimagnesiuretic effects of potassium-sparing diuretics, different mechanisms of action have already been suggested (Devane & Ryan, 1981; Ryan *et al.*, 1984; Ryan, 1986). Due to the differences between RPH 3036 and potassium-sparing diuretics most of these proposed mechanisms cannot account for the effects observed after administration of RPH 3036.

Magnesium-sparing properties of amiloride are thought to be mediated by direct renal action (Devane & Ryan, 1983). A blockade of sodium channels in the late distal tubule and in the collecting duct by potassium-sparing diuretics will result in a reduction of the transepithelial membrane potential

(Hendry & Ellory, 1988). This reduces the driving force for potassium exit into the lumen, and thus potassium excretion is attenuated. Some authors suggest (Ryan *et al.*, 1984) that a lower transepithelial potential in this part of the nephron might promote reabsorption of cations such as magnesium, too, thereby reducing their excretion.

This mechanism of action can explain only combined antidiuretic and antimagnesiuretic effects but it cannot explain a reduction of potassium excretion without reduction of magnesium excretion or selective magnesium-sparing effects. Therefore, the magnesium-retaining effects of RPH 3036 must be mediated by a different mode of action.

RPH 3036 (100 $\mu\text{mol kg}^{-1}$) only induced excretion of about 50% of the given saline load after 2.5 h (see Figure 3). Thus, it seems to be unlikely that magnesium reabsorption in the proximal tubule was increased due to a reduction in extracellular fluid volume.

The fact that calcium excretion was not influenced by RPH 3036 may be taken as a hint that parathyroid hormone and calcitonin are not involved in the antimagnesiuretic effect of RPH 3036.

Since the effects on magnesium excretion were already evident 2 h after administration of RPH 3036 (Figure 4), a contribution of changes in plasma aldosterone or plasma thyroid hormone concentration to these actions was also unlikely.

The attempt to explain magnesium-retaining effects with an unspecific alkalosis (Ryan, 1986) does not seem to be sufficient as an explanation for the marked and selective effects induced by RPH 3036.

The mode of action which is most consistent with the results of this study is the selective inhibition of magnesium secretion in the late distal tubule and collecting duct. Although it is not unequivocally accepted that magnesium secretion occurs in that part of the nephron (Massry *et al.*, 1969; Alfredson & Walser, 1970) there are hints that magnesium is secreted in the late distal tubule and collecting duct at least during furosemide and saline diuresis (Rios *et al.*, 1977; Lechene & Blouch, 1979). Since our study was performed in saline-loaded rats it is conceivable that magnesium was secreted in the late distal tubule and collecting duct and that blockade of this secretion resulted in a selective reduction of magnesium excretion. Of course further studies are required to determine whether this suggestion holds true.

In conclusion, RPH 3036 and RPH 3038, two recently synthesized pteridine derivatives, exerted in conscious saline-loaded rats natriuretic and antimagnesiuretic effects probably by direct renal action. Due to the fact that these compounds influenced magnesium but not potassium and calcium excretion they might be useful tools for the future investigation of renal magnesium transport.

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