Investigations on the Magnesium Retaining Properties of Triamterene Derivatives with an Aromatic Side Chain

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Zusammenfassung

In Diureseversuchen an Ratten wurden neutrale (N) und saure Ether (S) des 4-Hydroxytriamteren mit aromatischer Seitenkette hinsichtlich ihrer elektrolytausscheidenden Eigenschaften untersucht. Dabei konnten die für Triamterenderivate mit aliphatischer Seitenkette geltenden Struktur-Wirkungs-Beziehungen bestätigt werden. Ebenfalls bestätigt wurde als magnesiumsparendes Strukturelement eine sauerstoffhaltige funktionelle Gruppe, die nicht ionisierbar sein darf und sich in einem bestimmten räumlichen Abstand zum Triamterengrundkörper befinden muß. Die Empfindlichkeit dieses magnesiumretinierenden Strukturelements gegenüber sterischer Hinderung wurde nachgewiesen.
Aufgrund der vorgestellten Struktur-Wirkungs-Beziehungen besteht die Möglichkeit, kaliumsparende Diuretika mit zusätzlichen antimagnesiuretischen Eigenschaften zu konzipieren.

Summary

The influence of neutral (N) and acidic ethers (S) of 4-hydroxytriamterene with an additional aromatic substituent in the side chain on renal electrolyte excretion was investigated in rats. The same structure-activity-relationships concerning the natriuretic, antikaliuretic and antimagnesiuretic properties as found in experiments with triamterene derivatives containing an aliphatic side chain could be established. These experiments also substantiated a non-ionizable oxygen function as the structural element for a magnesium sparing effect. It could be shown that this moiety must have a defined distance to the heterocyclic nucleus of the molecule, steric hindrance decreasing the magnesium sparing properties.
In conclusion, these compounds can be considered as valuable tools for the development of potassium retaining diuretics with additional magnesium sparing properties.

Résumé

On a étudié l'effet d'éthers neutres et acides du 4-hydroxytriamtérène, dotés d'un substituant aromatique additionnel sur la chaîne latérale, sur l'excrétion rénale d'électrolytes chez le rat. Ces études ont montré la même relation entre la structure et l'effet natriurétique, antikaliurétique et antimagnésiurétique que pour les dérivés du triamtérène dotés d'une chaîne latérale aliphatique. Elles ont en outre prouvé que l'élément structurel responsable de l'effet d'épargne du magnésium était un groupement non ionisable contenant de l'oxygène. Il a été établi que cet élément devait se trouver à une distance précise du noyau hétérocyclique de la molécule et que l'inhibition stérique diminuait les propriétés d'épargne du potassium.
En conclusion, ces produits peuvent être considérés comme des outils précieux pour le développement de diurétiques d'épargne potassique dotés en même temps de propriétés d'épargne du magnésium.

Introduction

Early studies of triamterene analogues, in which the development of better water-soluble compounds was our main objective, have shown that some derivatives possess antikaliuretic as well as antimagnesiuretic properties in white wistar rats. This additional effect could be observed within 2.5 h after i.v. application [1].

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A non-ionizable oxygen function (e.g. a hydroxy-, ether-, carbonyl- or carboxamide group) in the aliphatic side chain of 4-hydroxytriamterene ethers was postulated to be responsible for the antimagnesiuretic effect [2].
In the meantime, this hypothesis could be corroborated with 4-alkyl derivatives of triamterene [3, 4].
In the present study the pharmacodynamic properties, especially the magnesium sparing effect, of neutral and acidic 4-hydroxytriamterene ethers with an additional aromatic ring in the side chain were investigated [4]. The structures of the tested compounds are shown in figure 1.

Methods

Male Wistar rats with a body-weight of 130–170 g were used for the experiments. They were kept in a climatized animal cage at 22 °C with a relative atmospheric humidity of 50 %; the rats received a standard laboratory diet (Altromin®) and tap water ad libitum. Food was withdrawn 18 h prior to the experiment but access to water was unrestricted.
For each study, the animals (n = 6) were randomly divided into the treatment groups. Under light ether anaesthesia all rats received 20 ml/kg body-weight of a 0.9 % sodium chlori-

de solution by gavage before the injection of 25 µmol/kg body-weight of the test compound in one of the caudal veins. 6 animals, serving as controls (K), were treated with the corresponding solvent (20 % polyethylene glycole 400 and 80 % water) only. The rats were placed into individual metabolism cages without food and water. After a collecting period of 2.5 h the urine volumes were measured and the concentrations of Na^+ and K^+ were determined by flame photometry, those of Mg^{++} by atom absorption using the Elektrolytautomat FL 6 (*Zeiss, Oberkochen*, GFR).

In the histograms, data are presented as means with standard errors of the mean (S.E.M.). Differences between mean values were tested for statistical significance by U-test of *Wilcoxon, Mann* and *Whitney* [5]. The differences between the findings were considered significant (*) at $p < 0.05$.

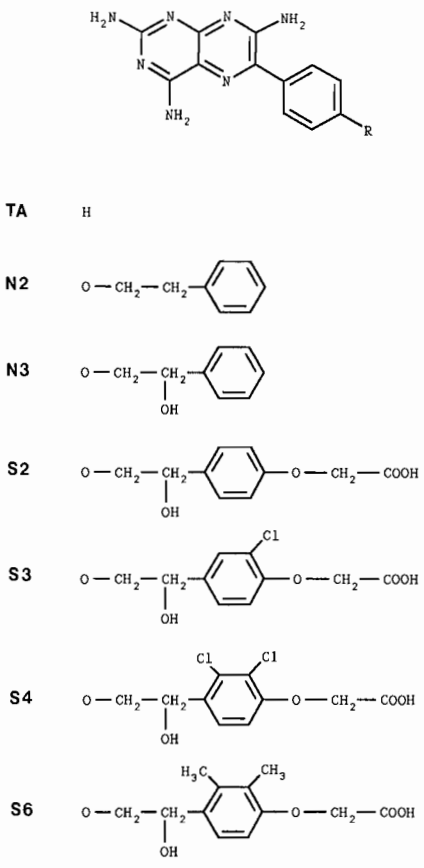

Fig. 1: Structural formulas of
Triamterene (TA)
4-(2-Phenylethoxy)triamterene (N2)
4-(2-Hydroxy-2-phenylethoxy) triamterene (N3)
4-(2-(4-Carboxymethoxyphenyl)2-hydroxyethoxy) triamterene (S2)
4-(2-(4-Carboxymethoxy-3-chlorophenyl)-2-hydroxyethoxy) triamterene (S3)
4-(2-(4-Carboxymethoxy-2,3-dichlorophenyl)-2-hydroxyethoxy) triamterene (S4)
4-(2-(4-Carboxymethoxy-2, 3-dimethylphenyl)-2-hydroxyethoxy) triamterene (S6)

Results

The urine and electrolyte excretion following intravenous application of triamterene and its derivatives are shown in fig. 2–4.

Triamterene, the neutral compounds N2 and N3 and the acidic derivatives S2 and S3 exhibited a diuretic and natriuretic effect. However, substitution with a carboxymethoxy group (S2, S3, S4 and S6) resulted in a marked decrease in diuretic and natriuretic activity compared to triamterene and the neutral derivatives.

The dichloro- and dimethyl-substituted phenoxyacid derivatives S4 and S6 proved to have a natriuretic and diuretic excretion comparable with control values. For all acidic compounds tested only a weak potassium retaining activity could be demon-strated.

In contrast, triamterene and the neutral derivatives N2 and N3 caused a marked decrease in potassium excretion. Additionally, compounds N3, S2 and S3 showed an anti-magnesiuretic effect.

For triamterene, N2 and the phenoxy acid compounds S4 and S6 no magnesium sparing properties could be found.

Discussion

In analogy to earlier studies with aliphatic triamterene derivatives, our results show that compounds with an additional aromatic ring in the side-chain of 4-hydroxytriamterene analogues may have the property of potent natriuretic and potassium retaining substances. As expected, an acidic functional moiety in the side-chain led to compounds with a diminished potassium retaining potency [6]. Furthermore, for acidic derivates with a disubstituted phenyl ring (S4, S6) a marked decrease in natriuretic activity was shown. We conclude that natriuresis was reduced by the influence of steric hindrance [7].

As noted before, triamterene derivatives with a non-ionizable oxygen function in the ether side-chain should

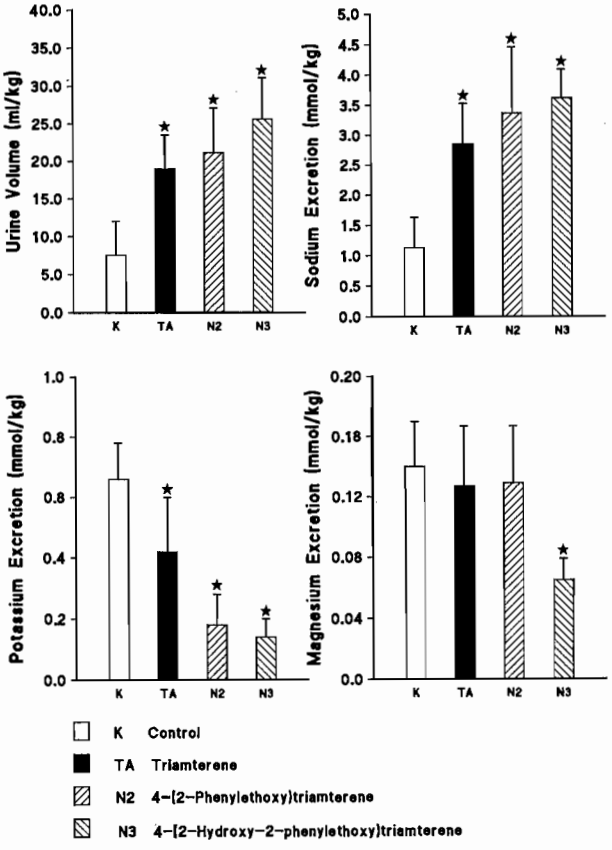

Fig. 2: Urinary and electrolyte excretion after intravenous application of 25 µmol/kg TA, N2, N3.

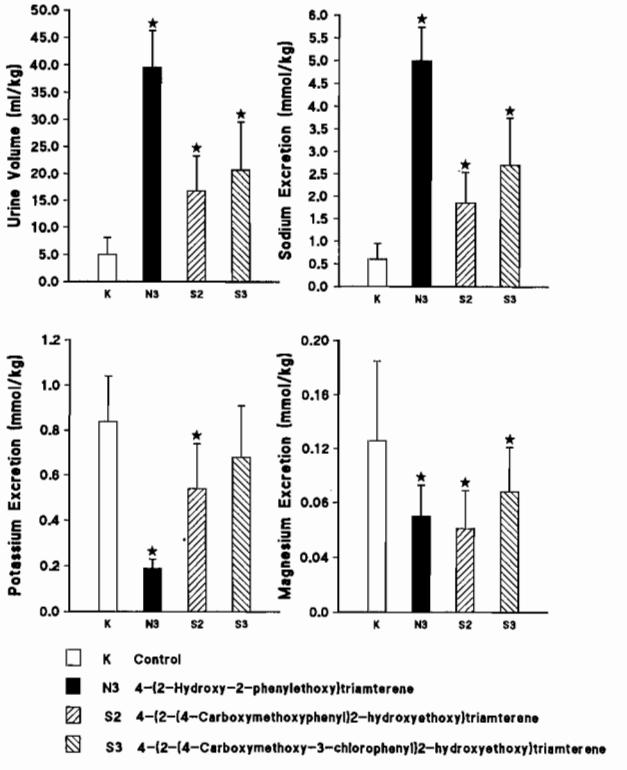

Fig. 3: Urinary and electrolyte excretion after intravenous application of 25 µmol/kg N3, S2, S3.

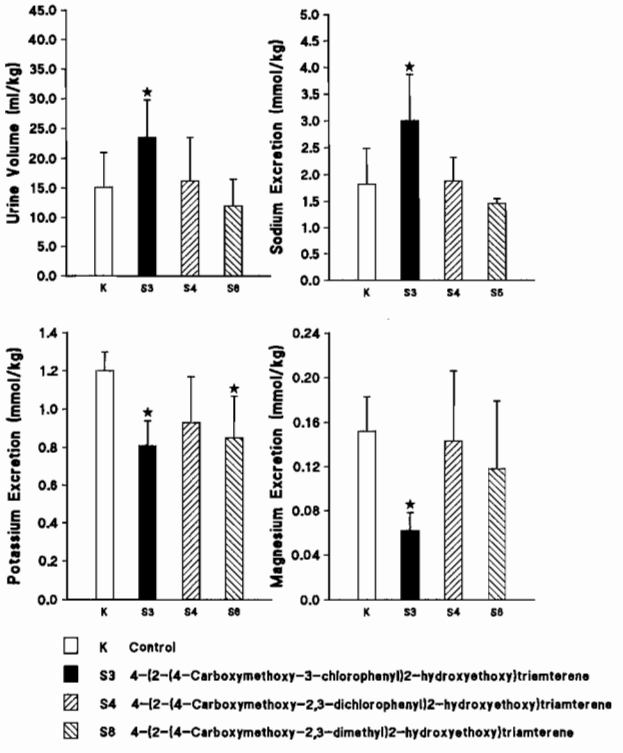

Fig. 4: Urinary and electrolyte excretion after intravenous application of 25 µmol/kg S3, S4, S6.

exhibit a magnesium retaining activity [2], too.

For compound N3 with a neutral and compounds S2 and S3 with an acidic side-chain and an additional hydroxy group an antimagnesiuretic effect could be demonstrated. In contrast, the disubstituted compounds S4 and S6 with a hydroxy group did not show any magnesium sparing effect. A decrease in magnesium retaining activity was already conspicious for the monosubstituted derivative S3. It is therefore concluded that steric hindrance of the "magnesium retaining" hydroxy group by the chloro atoms or the methyl groups is responsible for the loss in magnesium retaining efficacy. These findings are in agreement with studies carried out with neutral and basic aliphatic triamterene derivatives. It could be demonstrated that replacement of a methyl group attached to the carbon atom with an oxygen function by a phenyl ring led to a marked decrease in maximum magnesium retaining effect (intrinsic efficacy) [4,8]. The same is true for basic derivatives. The low intrinsic efficacy with respect to magnesium retention of basic triamterene derivatives could be referred to steric hindrance of the hydroxy group by ionised and hydrated amino groups in α-position [1].

To summarize, the experimental data derived from these studies with triamterene derivatives with an additional aromatic ring in the side-chain

- confirm structure-activity-relationships of aliphatic triamterene analogues with regard to natriuresis and antikaliuresis.
- suggest that a non-ionizable oxygen in a defined distance to the heterocyclic nucleus of the molecule [4] or as a part of a flexible side chain [1] is responsible for the magnesium retaining effect. This group may interact with special structures of an ion-channel (e.g. hydrogen bond with the hydroxy group of the amino acid serine [9]) in the late distal nephron.
- show that steric hindrance of the oxygen function leads to a decrease or loss in magnesium retaining intrinsic efficacy.
- demonstrate that all magnesium retaining compounds tested possess potassium retaining properties, too. Therefore, we conclude that magnesium retention of triamterene derivatives is coupled with the antikaliuretic property [8].

Acknowledgement

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