# Investigations on the Antidiuretic Activity of Some Potassium and Magnesium Retaining Triamterene Derivatives in Rats

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## Zusammenfassung

Beim Screening kalium- und magnesiumretinierender Triamterenderivate an Ratten wurde bei einigen Substanzen in höherer Dosierung eine antidiuretische Wirkung beobachtet. Durch diesen Effekt können Fehlinterpretationen bei der Bewertung kalium- und magnesiumretinierender Eigenschaften von Triamterenderivaten auftreten. Zusammenhänge zwischen der antidiuretischen Wirkung und der Molekülstruktur, der akuten Toxizität und der Löslichkeit der Derivate wurden untersucht. Konsequenzen für das Screening von kalium- und magnesiumretinierenden Triamterenderivaten an Ratten werden beschrieben.

## Summary

During screening of potassium and magnesium retaining triamterene derivatives in conscious saline-loaded rats an antidiuretic effect was observed with higher doses of some compounds. This effect may lead to misinterpretations concerning the potassium and magnesium retaining properties of these substances. The interrelationship between the antidiuretic activity and the molecular structure, the acute toxicity and the solubility of these derivatives was investigated. Consequences for the screening of potassium and magnesium retaining substances in rats are shown.

## Résumé

Au cours de l'étude des dérivés de triamtérène épargnant le potassium et le magnésium chez le rat conscient chargé en sel, on a observé un effet antidiurétique avec des doses plus fortes de certains composés. Cet effet peut entrainer une mauvaise interprétation concernant les propriétés d'épargne du potassium et du magnésium de ces substances. On a étudié les relations mutuelles entre l'activité antidiurétique et la structure moléculaire, la toxicité aiguë et la solubilité de ces dérivés. On indique les conséquences sur la sélection des substances épargnant le potassium et le magnésium chez le rat.


## Introduction

Compounds with bell-shaped dose-response relationships exhibit a downturn of response when a certain dose is exceeded [1]. During screening of triamterene derivatives for antikaliuretic and antimagnesiuretic properties in conscious saline-loaded rats some compounds showed this effect with a reduction of the initially increased urine and sodium excretion with rising doses, whereby the potassium and magnesium excretion decreased, too [4, 7, 13]. Since this antidiuretic activity induced by different triamterene derivatives was well reproducible it appears to be a specific effect [4]. In these cases no conclusions can be drawn concerning the antikaliuretic and antimagnesiuretic properties of triamterene derivatives in rats since the potassium and magnesium retaining activity cannot be distinguished from that mediated by an antidiuretic effect. Thus, the aim of this study was to further investigate this effect and to evaluate the reason for its occurrence. The results of the dose-response experiments were compared with the molecular structure, the acute toxicity and the solubility of the substances. The structures of the compounds investigated are shown in fig. 1. They are all derivatives of 4'-hydroxy-triamterene.

## Material and Methods

Compounds A4 and A6 were synthesized by *Ullrich* [6] in the laboratories of Röhm GmbH, Darmstadt/Germany, the other compounds were kindly supplied by Procter and Gamble Pharmaceuticals-Germany GmbH, Weiterstadt/Germany.

Male Wistar rats with a body weight of 130–170g were used for the experiments [4]. They were kept in a climatized animal cage at 22°C with a relative humidity of 50%; the rats received a standard laboratory diet (Altromin R) and tap water ad libitum. Food was withdrawn 18h prior to the experiment but access to water was unrestricted.

For each study, the animals were randomly divided into the treatment groups. Before the intravenous application all rats received 20ml/kg body weight of a 0.9% sodium chloride solution by gavage. Under light ether anaesthesia the test compound was injected in one of the caudal veins. For the evaluation of the dose-response curves 9–11 doses between 0.01μmol/kg and 100μmol/kg body weight were applied to 2–4 animals each. 4–5 animals, serving as controls, were treated with the corresponding solvent (20% polyethylene glycole 400 in water) only. The rats were placed into individual metabolism cages without food and water. After a collecting period of 2.5h


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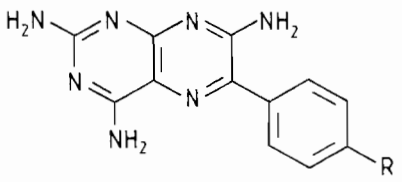
the urine volumes were measured and the concentrations of $Na^+$ and $K^+$ were determined by flame photometry, those of $Mg^{2+}$ by atom absorption using the Elektrolytautomat FL 6 (Zeiss, Oberkochen/Germany).

To describe the dose-response relationships the following equation was chosen [19]: $E = E_0 + E_{max} \cdot [D^P/(D^P + ED_{50}^P)]$, where D is the applied dose, E is the effect observed after application of D, $E_0$ is the basic excretion of urine volume or ions in the control group (D = 0), $E_{max}$ is the maximal change of urine volume or ion excretion that can be achieved by the test compound (= a measure of the efficacy), and $ED_{50}$ is the dose of the tested drug which produces a half-maximal effect (= a measure of the potency); P allows correction for variable slopes of dose-response curves. The course of the logistical function for urine volume and sodium excretion is shown in fig 2, for potassium and magnesium excretion in fig. 3. The coefficients of the model function were fitted to the data of urine volume, $Na^+$, $K^+$ and $Mg^{2+}$ excretion by nonlinear least-square regression analysis using the program NONLIN [5].



R

A 1 –H

A 2 $-O-CH_2-CH_3$

A 3 $-O-CH_2-CH_2-OH$

A 4 $-O-CH_2-CHOH-CH_3$

A 5 $-O-CH_2-CHOH-CH_2-N(CH_3)_2$

A 6 $-O-CH_2-C(=O)-CH_3$

A 7 $-O-CH_2-C(=O)-OH$

A 8 $-O-(CH_2)_4-C(=O)-OH$

A 9 $-O-S(=O)(=O)-OH$

Fig. 1: Structural formulas of the tested compounds and abbreviations used: A1 – Triamterene, A2 – 4-Ethoxytriamterene, A3 – 4-(2-Hydroxyethoxy)triamterene, A4 – 4-(2-Hydroxypropoxy)triamterene, A5 – 4-(3-Dimethylamino-2-hydroxypropoxy) triamterene, A6 – 4-(2-Oxopropoxy)triamterene, A7 – 4-Carboxymethoxytriamterene, A8 – 4-(4-Carboxybutoxy)triamterene, A9 – 4-Hydroxytriamterene sulfuric acid ester.

## Results and Discussion

The fitted parameters $E_0$, $E_{max}$, $ED_{50}$ and P for urine volume, sodium, potassium and magnesium excretion calcu-

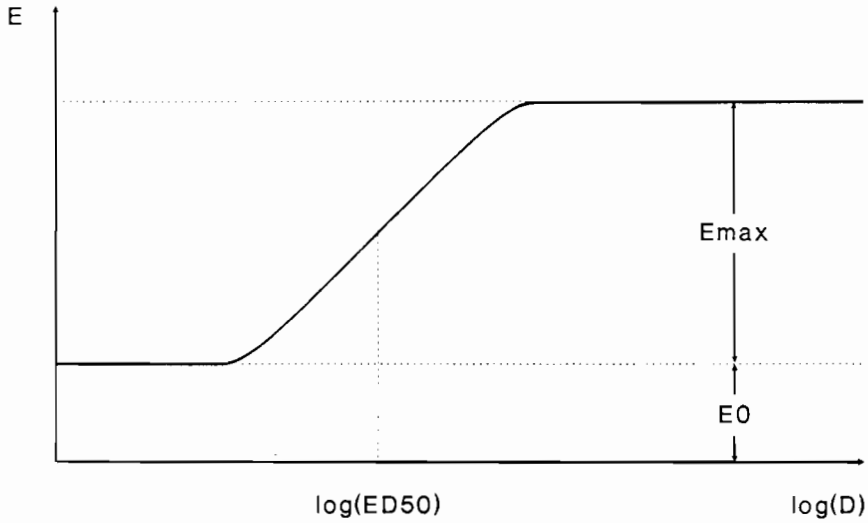



Fig. 2: Dose-response curve for urine volume and sodium excretion (D is the applied dose, E is the effect observed after application of D, $E_0$ is the basic excretion of urine volume or $Na^+$ in the control group [D = 0], $E_{max}$ is the maximal change of urine volume or $Na^+$ excretion that can be achieved by the test compound and $ED_{50}$ is the dose of the tested drug which produces a half-maximal effect).

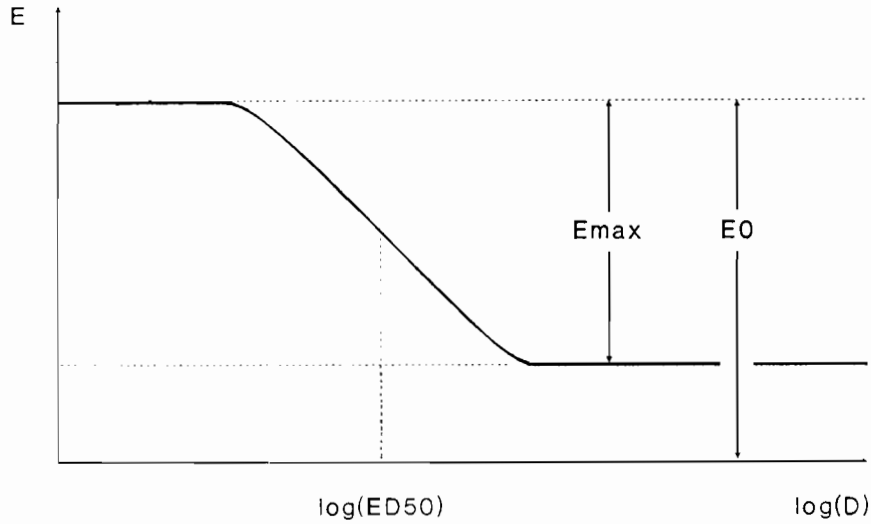



Fig. 3: Dose-response curve for potassium and magnesium excretion (D is the applied dose, E is the effect observed after application of D, $E_0$ is the basic excretion of $K^+$ or $Mg^{2+}$ in the control group [D = 0], $E_{max}$ is the maximal change of $K^+$ or $Mg^{2+}$ excretion that can be achieved by the test compound and $ED_{50}$ is the dose of the tested drug which produces a half-maximal effect).

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Tab. 1: Fitted coefficients of the model function describing the dose-dependent excretion of urine volume, sodium, potassium and magnesium of the tested compounds (B: bell-shaped dose-response relationship).

| | Urine volume | | | | Sodium | | | | Potassium | | | | Magnesium | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $ED_{50}$ ($\mu$mol/kg) | $E_0$ (ml/kg) | $E_{max}$ (ml/kg) | P | $ED_{50}$ ($\mu$mol/kg) | $E_0$ (mmol/kg) | $E_{max}$ (mmol/kg) | P | $ED_{50}$ ($\mu$mol/kg) | $E_0$ (mmol/kg) | $E_{max}$ (mmol/kg) | P | $ED_{50}$ ($\mu$mol/kg) | $E_0$ (mmol/kg) | $E_{max}$ (mmol/kg) | P |
| A1 | 3.73 | 5.95 | 10.52 | 2.93 | 4.40 | 1.02 | 1.54 | 20.52 | 1.87 | 0.201 | −0.146 | 1.17 | | | | |
| A2 | 25.53 | 8.25 | 12.15 | 1.78 | 10.01 | 0.93 | 2.27 | 1.34 | 5.58 | 0.396 | −0.367 | 2.23 | | | | |
| A3 | B | B | B | B | B | B | B | B | | | | | | | | |
| A4 | 9.70 | 9.08 | 18.98 | 2.72 | 8.79 | 1.89 | 2.97 | 1.81 | 4.70 | 0.706 | −0.449 | 2.64 | 7.29 | 0.148 | −0.113 | 0.48 |
| A5 | 5.81 | 4.31 | 20.46 | 4.08 | 9.36 | 1.11 | 3.83 | 1.49 | 0.24 | 0.259 | −0.203 | 1.74 | 0.40 | 0.101 | −0.038 | 4.71 |
| A6 | 9.55 | 8.49 | 17.81 | 2.62 | 8.17 | 1.39 | 2.75 | 2.46 | 8.50 | 0.659 | −0.634 | 0.54 | 13.61 | 0.120 | −0.077 | 1.52 |
| A7 | B | B | B | B | B | B | B | B | | | | | | | | |
| A8 | 4.51 | 3.87 | 22.54 | 8.22 | 5.86 | 1.37 | 3.32 | 17.19 | 20.63 | 0.280 | −0.220 | 28.21 | | | | |
| A9 | B | B | B | B | B | B | B | B | | | | | | | | |

lated from the measured data after application of the compounds tested are shown in tab. 1. Complete dose-response curves could be obtained for the substances A1, A2, A4, A5, A6 and A8. In contrast, the compounds A3, A7 and A9 showed an antidiuretic effect in the conscious saline-loaded rat after intravenous administration of higher doses. Consequently, for these derivatives bell-shaped dose-response curves were obtained and the corresponding coefficients could not be calculated.

Initially, with rising doses of A3, A7 and A9 urine volume and sodium excretion increased. The potassium and magnesium excretion were influenced as predicted by the structure of the compounds [2, 19]. However, with further dose elevation urine and sodium excretion decreased, so that they finally equalled the values of the control group or were even slightly lower. In contrast, the potassium and magnesium excretion showed always a continuous decrease. The antidiuretic phase started for all compounds at dosages about 25$\mu$mol/kg [4]. During the whole collecting period of 2.5h the animals behaved normally just as the control group.

A7 and A9 are triamterene derivatives with an acidic side-chain whereas the compound A3 has a neutral side-chain. In the rat A7 and A9 are metabolically stable compounds [9, 10]. The metabolism of A3 is unknown. Interestingly, only triamterene derivatives with acidic or neutral side-chain exerted the depressing effects on diuresis and saluresis. Ethers of 4'-hydroxytriamterene with a basic side-chain (see A5), which are hardly metabolized [9], this effect did not show [3, 12]. A3, A7 and A9 have in common that they possess short side-chains with electronegative atoms (O, S) and high electron density close to the phenyl moiety of the triamterene molecule (fig. 1). This chemical property seems to be characteristic for the antidiuretic activity of these compounds. An increase in the number of C-atoms resp. a reduction of the number of electronegative oxygen atoms in the side-chain abolishes the antidiuretic effect (see A3/A4, A3/A2, A7/A6, A7/A8).

Comparing the $LD_{50}$-values (after intravenous single dose administration in mice) of A7 (462$\mu$mol/kg) and A9 (> 572$\mu$mol/kg) it can be shown that the antidiuretic effect starts far below these dosages [18]. While triamterene (A1) has a higher acute toxicity (99$\mu$mol/kg) than its metabolite 4'-hydroxytriamterene sulfuric acid ester (A9), only this metabolite exerts an antidiuretic effect. In addition, A7 and A8 (699$\mu$mol/kg) have about the same $LD_{50}$-value, but only A7 is antidiuretically active. A5 with an $LD_{50}$-value of 59$\mu$mol/kg and other triamterene derivatives with a basic side-chain and comparable $LD_{50}$-values does not show this effect, too [3]. These findings suggest that the antidiuretic effect of triamterene derivatives does not correlate with their acute toxicity.

The solubility (in phosphate buffer, pH 7.4) of triamterene and its derivatives with neutral and acidic side chains investigated was found to be between 5$\mu$mol/l (A2) [16] and 190$\mu$mol/l (A4) [6]. Compound A5 with a basic side chain is an exception with a solubility of 703 $\mu$mol/l [15]. Generally, the solubility of the compounds with neutral and acidic side-chains is lower than that of the triamterene derivatives with a basic side-chain. The comparison of the compounds with neutral side-chains A2 and A3 (10$\mu$mol/l) [16] as well as that of the acidic compounds A7 (40$\mu$mol/l) [13] and A8 (6$\mu$mol/l) [15] shows that the compound with the better solubility exerts an antidiuretic effect. The same was found to be true for triamterene (A1, 83$\mu$mol/l) [6] and its metabolite 4'-hydroxytriamterene sulfuric acid ester (A9, 129$\mu$mol/l) [13] which is better soluble than triamterene and is antidiuretically active. On the other hand, both A9 and 2,4,7-triamino-6 - ( 4' - methanesulfon - amidophenyl ) pteridine which has a solubility of 10$\mu$mol/l) exert an antidiuretic effect [7] although their solubilities differ about 10 times. 4'-methanesulfonamidotriamterene shows an antidiuretic effect at dosages greater than 10$\mu$mol/kg, has NH-acidic properties [17], and fits with its molecular structure in our proposed concept. In addition, this compound did not form any histologically visible deposits in the kidney up to the highest dose (100 $\mu$mol/kg) which was used [7]. These facts suggest that formation of crystalline deposits of triamterene derivatives in the kidney [14] is an insufficient explanation for the antidiuretic effect.

Neither acute toxicity nor low solubility seem to be the reason for the observed decline in renal function. Therefore, it appears that this effect is mediated by a structure-specific pharmacodynamic action of some triamterene derivatives.

# Investigations on the Antidiuretic Activity of Some Potassium and Magnesium Retaining Traimterene Derivatives in Rats

To avoid misinterpretations concerning the antikaliuretic or antimagnesiuretic effect of triamterene derivatives in conscious saline-loaded rats, it is necessary to perform complete dose-response curves including not only potassium and magnesium excretion but also urine volume and sodium excretion.

Since an antidiuretic effect of triamterene derivatives has yet been investigated only in the rat its clinical relevance remains unclear. However, the main metabolite of triamterene in humans [8], the 4'-hydroxytriamterene sulfuric acid ester (A9), exerts in contrast to triamterene itself antidiuretic activity in rats. Therefore, this metabolite may be involved in the occurrence of side effects in humans such as acute renal failure observed during combination therapy of triamterene with non-steroidal antiinflammatory drugs [11].

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