Contribution to the Mode of Action of Potassium and Magnesium Retaining Triamterene Derivatives*

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Zusammenfassung

In der vorliegenden Arbeit werden Struktur- und Dosis-Wirkungsbeziehungen kalium- und magnesiumsparender Triamterenderivate beschrieben.
Zur Erklärung der Ergebnisse wird ein erweitertes Modell zum Wirkungsmechanismus von Triamteren und seinen Derivaten vorgeschlagen. Dabei wird von drei unterschiedlichen Transportsystemen für Na^+, K^+ und Mg^{2+} ausgegangen. Durch den Angriff bestimmter Triamterenderivate an diesen Transportsystemen wird die Resorption von Na^+ sowie die damit spezifische gekoppelte Sekretion von K^+ und Mg^{2+} im distalen Tubulus und Sammelrohr der Niere verhindert.

Summary

In the present study structure-activity- and dose-response-relationships of potassium- and magnesium-retaining triamterene derivatives are described.
To explain the findings an extended model of the action pattern of triamterene and its derivatives is proposed. There are three different transport systems for Na^+, K^+ and Mg^{2+}. Na^+ absorption and with it specific coupled K^+ and Mg^{2+} secretion in the late distal tubule and collecting duct of the nephron is hindered by triamterene derivatives by blockade of these transport systems.

Résumé

La présente étude decrit les relations structure-activité et dose-réponse propres aux dérivés du triamtérène épargneurs de potassium et de magnésium.
Nous proposons un modèle élargi du mécanisme d'action du triamtérène et de ses dérivés afin d'expliquer les résultats. Il existe 3 systèmes différents de transport du Na^+, du K^+ et du Mg^{2+}. Par blocage de ces systèmes de transport, les dérivées du triamtérène empêchent l'absorption du Na^+ ainsi que la secrétion couplée spécifique du K^+ et du Mg^{2+} dans le tube contourné distal et le tube collecteur.

Introduction

Beside its potassium-retaining properties the diuretic agent triamterene was found to possess a magnesium retaining effect. In contrast to its antikaliuretic effect, the magnesium retention produced by triamterene is only weak and the onset of action is not observed immediately after dosing but appears several hours afterwards [1, 2, 8, 17].
For several triamterene derivatives it could be demonstrated that they exert a marked antimagnesiuresis only a short time after application [3]. This effect depends on the substitution of the side-chain of the derivatives [4, 5]. Early work concerning structure-activity-relationships of triamterene derivatives showed that the sodium and potassium excretion of these compounds is highly sensitive to variations of their molecule structure, too [6, 7, 8, 11, 14].
In addition to these investigations it was the aim of the present study to establish dose-response-relationships of triamterene derivatives, in respect to the Mg^{2+} excretion compared with the Na^+ and Ka^+ excretion and to discuss the mode of nephronal action of these compounds.
The structures of the compounds tested are shown in fig. 1 (P = only potassium retaining activity, M = potassium and magnesium retaining activity).

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*Dedicated to Prof. Dr. *Mutschler*, E. on the occasion of his 60th birthday.

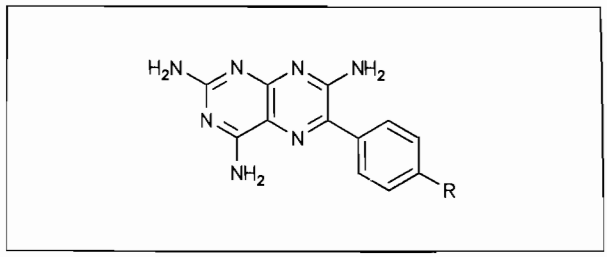
Materials and Methods

Materials

Compounds M3–M8 were synthesized by *F. Ullrich* in the laboratories of Röhm GmbH, Darmstadt, GFR, the other compounds were kindly supplied by Röhm Pharma, Weiterstadt, GFR.

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 standard laboratory diet



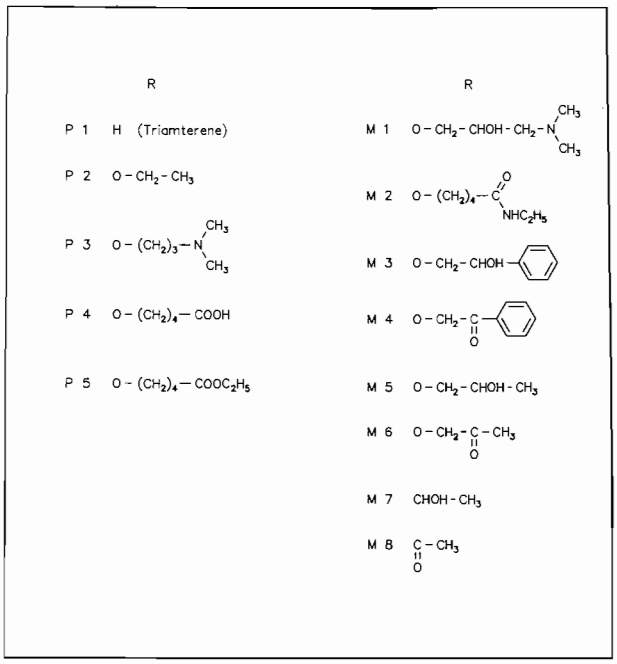


Fig. 1: Structural formulas of tested compounds and abbreviations used:

P1	Triamterene
P2	4-Ethoxytriamterene
P3	4-(3-Dimethylaminopropoxy)triamterene
P4	4-(4-Carboxybutoxy)triamterene
P5	Ethylester of P4
M1	4-(3-Dimethylamino-2-hydroxypropoxy)triamterene
M2	Ethylamide of P4
M3	4-(2-Hydroxy-2-phenylethoxy)triamterene
M4	4-(2-Oxo-2-phenylethoxy)triamterene
M5	4-(2-Hydroxypropoxy)triamterene
M6	4-(2-Oxopropoxy)triamterene
M7	4-(1-Hydroxyethyl)triamterene
M8	4-Acetyltriamterene

(Altromin®) 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 20 ml/kg bodyweight 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 0.01 and 100 µmol/kg bodyweight 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 2.5 h 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, GFR).

To describe the dose-response-relationships the following equation was chosen [9]:

$$E = E_0 + E_{max}[D^P/(D^P + ED_{50}^P)]$$

where D is the applied dose, E is the effect observed after the application of D, E_0 is the basic excretion of ions in the control group (D = 0), E_{max} is the maximal change of ion excretion that can be achieved by the test compound (= a measure of the intrinsic efficacy), and ED_{50} is the dose of the tested drug which produces a half-maximal effect (= a measure of the potency); P allows for variable slopes of the dose-response curves.

The course of the logistical function for the sodium- and for the potassium- and magnesium excretion is shown in fig. 2 and 3.

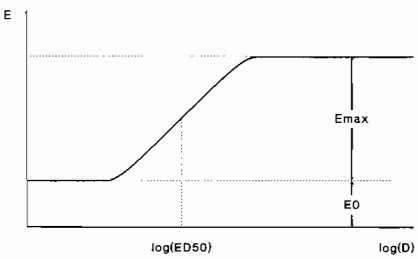


Fig. 2: Dose-response curve for sodium excretion.

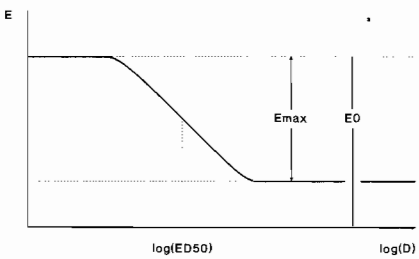


Fig. 3: Dose-response curve for potassium and magnesium excretion.

The coefficients of the model function were fitted to the data of urinary Na^+, K^+ and Mg^{2+} excretion by nonlinear least square regression analysis using DEC 10 and Apple IIe computers [10].

Results

The fitted parameters E_0, E_{max}, ED_{50} and P of sodium, potassium and magnesium excretion for all tested compounds are shown in tab. 1 and fig. 4–8 [8, 11, 13].

In fig. 4, the logarithms of the values calculated for ED_{50} of the sodium excretion against the logarithms of the values calculated for ED_{50} of the potassium retention are shown.

While the ED_{50} values of all tested compounds for the sodium excretion were in the same range, the ED_{50} values for the potassium retention differed up to two orders of magnitude. Derivatives with a basic side-chain

Tab. 1: Fitted coefficients of the model function describing the dose-dependent natriuretic, antikaliuretic and antimagnesiuretic effects of the tested compounds.

	Sodium				Potassium				Magnesium			
	ED_{50} (µmol/kg)	E_0 (mmol/kg)	E_{max} (mmol/kg)	P	ED_{50} (µmol/kg)	E_0 (mmol/kg)	E_{max} (mmol/kg)	P	ED_{50} (µmol/kg)	E_0 (mmol/kg)	E_{max} (mmol/kg)	P
P1	4.40	1.02	1.54	20.52	1.87	0.201	-0.146	1.17				
P2	10.01	0.93	2.27	1.34	5.58	0.396	-0.367	2.23				
P3	5.53	0.73	4.45	0.98	0.54	0.478	-0.332	1.16				
P4	5.86	1.37	3.32	17.19	20.63	0.280	-0.220	28.21				
P5	10.34	0.91	2.10	2.19	25.75	0.234	-0.222	1.07				
M1	9.36	1.11	3.83	1.49	0.24	0.259	-0.203	1.74	0.40	0.101	-0.038	4.71
M2	3.50	1.18	4.09	1.46	2.61	0.285	-0.260	1.41	3.68	0.101	-0.071	2.15
M3	5.54	1.17	3.22	2.58	2.88	0.601	-0.487	1.05	10.76	0.113	-0.049	6.50
M4	8.32	0.88	4.44	2.08	3.36	0.512	-0.398	1.34	4.44	0.096	-0.037	3.08
M5	8.79	1.89	2.97	1.81	4.70	0.706	-0.449	2.64	7.29	0.148	-0.113	0.48
M6	8.17	1.39	2.75	2.46	8.50	0.659	-0.634	0.54	13.61	0.120	-0.077	1.52
M7	11.97	1.17	2.36	1.53	9.41	0.650	-0.464	2.06	17.71	0.124	-0.067	2.56
M8	8.10	1.01	2.25	2.36	8.11	0.692	-0.305	0.73	10.57	0.133	-0.088	2.05

proved to be potent potassium sparing agents whereas compounds with an acidic moiety or an ester group [12] in the side-chain exhibit only weak potassium retaining properties. The values of the neutral compounds are ranging in between [8].

In fig. 5, the E_{max} values of the sodium excretion are plotted against the E_0 values, respectively. It becomes obvious that the E_{max} values for the sodium excretion are not altered by the control values (E_0). The maximum of the Na^+ excretion (E_{max}) is independent of E_0 and therefore a compound characteristic.

In contrast, there is a high correlation between E_0 values and E_{max} values for the potassium retention which indicates that all tested compounds have the same antikaliuretic intrinsic efficacy (fig. 6) [9].

The same holds true for magnesium-retaining properties of the triamterene derivatives investigated (fig. 7). Because of the high correlation all tested agents must be considered to possess the same antimagnesiuretic intrinsic efficacy.

In addition to the finding that triamterene derivatives with magnesium-retaining properties are at the same time antikaliuretics [5], it becomes

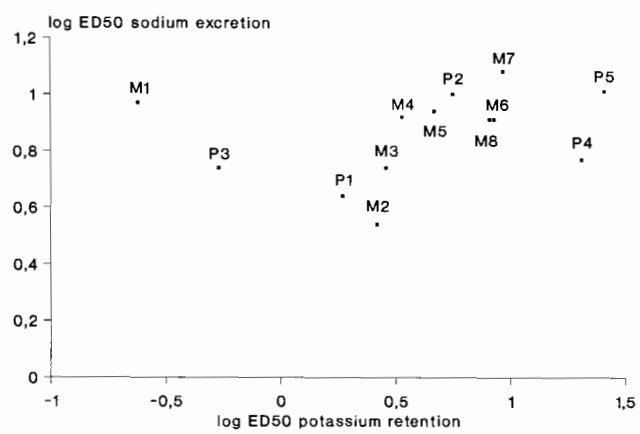


Fig. 4: Logarithms of the values calculated for ED_{50} of the sodium excretion plotted against the logarithms of the values calculated for ED_{50} of the potassium retention.

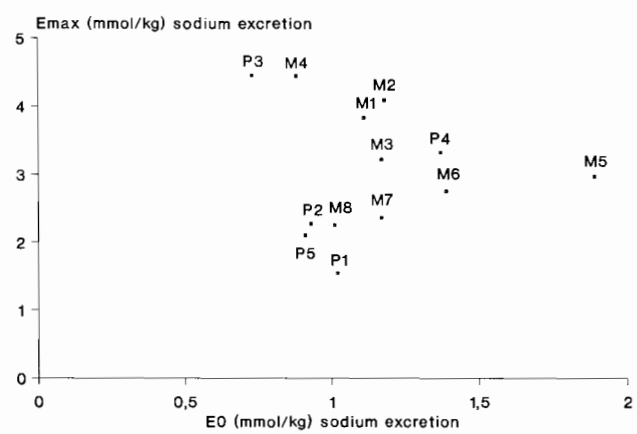


Fig. 5: Values calculated for E_{max} of the tested compounds on sodium excretion plotted against the corresponding values calculated for E_0.

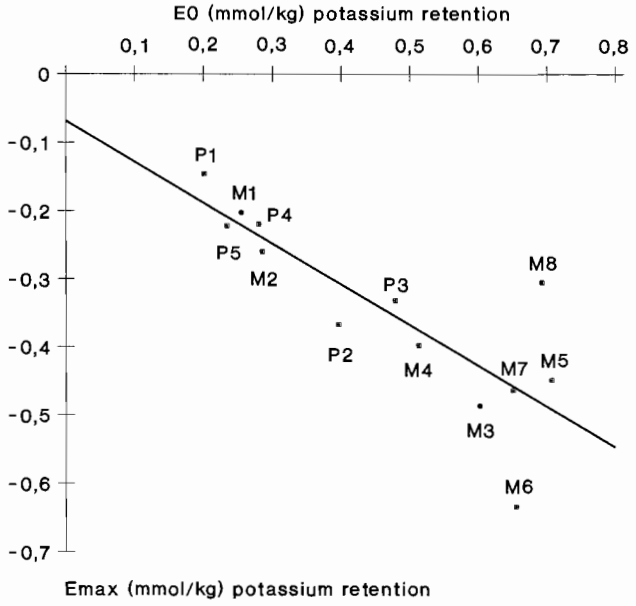


Fig. 6: Values calculated for E_{max} of the tested compounds on potassium retention plotted against the corresponding values calculated for E_0 (y = –0.6x – 0.07 r = 0.825).

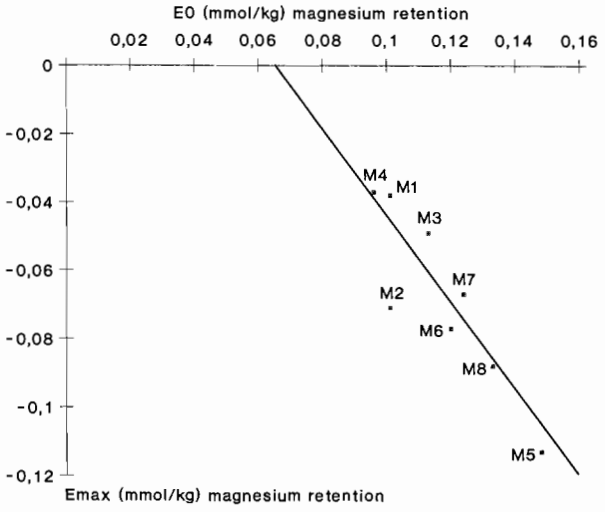


Fig. 7: Values calculated for E_{max} of the tested compounds on magnesium retention plotted against the corresponding values calculated for E_0 (y = –1.29x + 0.063 r = 0.888).

obvious from fig. 8 that there even exists a good correlation between the potassium and magnesium retaining potency [3].

Discussion

The pharmacological activity of potassium retaining diuretics (triamterene, amiloride) is explained by a reversible blockade of epithelial Na^+ channels in the late distal tubule and the collecting duct of the kidney [15, 23, 25, 31].

Blockade of the depolarizing Na^+ conductance by triamterene results in a reduced reabsorption of Na^+ accompanied with a diminished K^+ secretion. The transepithelial lumen-negative electrical potential difference disappears and at the same time the absorbtion of divalent Mg^{2+} should increase [15, 24, 25] or their secretion should decrease [22, 25, 30]. Based on this concept the pharmacodynamic effects of triamterene concerning the electrolyte excretion could be explained. However, the pharmarcological profiles of the tested triamterene derivatives with marked natriuretic, antikaliuretic and antimagnesiuretic properties cannot be described in detail by this model.

To explain the pharmacodynamic effects of the tested compounds we therefore postulate three different sites of action:

1. a Na^+ transport system
2. a K^+ transport system
3. a Mg^{2+} transport system

The claims for the structure of triamterene derivatives with regard to their specifity for these transport systems increases in the following sequence: Na^+-, K^+-, Mg^{2+}-, transport system.

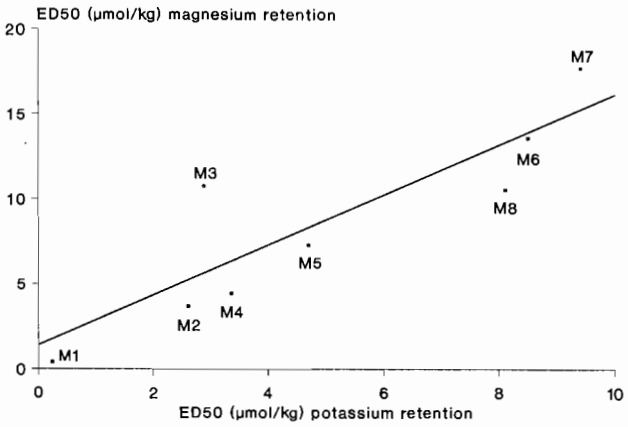


Fig. 8: Values calculated for ED_{50} of the tested compounds on magnesium retention plotted against the values calculated for ED_{50} on potassium retention (y = 1.25x + 0.98 r = 0.887).

1. Na^+ Transport System

Blockade of this system results in an inhibition of Na^+ absorption and increases its excretion. Because sodium excretion is nearly ten fold higher than K^+ and Mg^{2+} retention [11, 13], this transport system is responsible for the main part of Na^+ elimination. All tesodium excretion there is no dependence of E_{max} and E_0 (fig. 5).

2. K^+ Transport System

Interaction of triamterene derivatives with this transport system leads to a decrease of K^+ secretion. Basic analogues have a high, acidic compounds a low affinity to this system. The potency of neutral derivatives is ranging in

between (fig. 4). Therefore, it is concluded that potassium sparing triamterene derivatives interact with acidic structures of this transport system [27]. There is a strong correlation between E_0 and E_{max} values for the potassium retention of all tested substances (fig. 6). It is likely that there are no differences in the efficacies, although the calculated E_{max} values differ. The differences in E_{max} may be explained by variations of the K^+ excretion of the controls (E_0).

3. Mg^{2+} Transport System

This system regulates the magnesium excretion in the late distal nephron and collecting duct of the kidney. Blokkade of this transport system decreases Mg^{2+} excretion.

As only triamterene derivatives with an non-ionizable oxygen function in the side-chain possess affinity to this system, it can be concluded that these compounds show hydrogen-bonding interaction with specific structures of this transport system [5]. The correlation of the ED_{50} values for the Mg^{2+} and K^+ retention (fig. 8) points also at acidic structures as postulated for the K^+ transport system.

The same dependence of E_{max} and E_0 as shown for the potassium-retaining intrinsic efficacy could be demonstrated for the magnesium retention. But compared to the E_{max} values for K^+ retention the corresponding values for the magnesium-retaining efficacy are much lower [11, 13, 17]. Moreover, magnesium excretion cannot undergo a certain value (fig. 7).

It becomes obvious from these dose-responseinvestigations that there is no dependence between total amount of Na^+ excretion and those of K^+ or Mg^{2+} retention. In contrast, we found a lot of similiarities for potassium and magnesium retention. It ist therefore concluded that the transport mechanisms for K^+ and Mg^{2+} work in a comparable fashion because of comparable sites of action [3, 19, 20, 21].

The mode of action of potassium and magnesium retaining triamterene derivatives concerning the three transport systems can be best explained on the basis of different Na^+ channels. Whereas the Na^+ transport system only consists of Na^+ channels [32, 33], the K^+ [29, 35] and the Mg^{2+} transport system [16, 28] may be build up of Na^+ channels and corresponding K^+ and Mg^{2+} channels. Therefore, potassium and magnesium transport should be coupled with sodium transport.

Compared to possible alternatives (e.g. direct blockade of potassium and magnesium channels) the advantage of the proposed model is that all effects on electrolyte excretion could be explained only by the reversible inhibition of different sodium channels of the three transport system by triamterene derivatives [34, 36]. In addition, the potential difference of the luminal membrane as the main driving force for K^+ and Mg^{2+} transport could be confirmed [26].

Further studies, however, have to be carried out to substantiate this proposed model and to develop highly specific electrolyte transport inhibitors.

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