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Research Article

Pharmacodynamics of Basic Triamterene Derivatives

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Triamterene derivatives with a basic side chain and with an additional hydroxyl group in their side chain (hydroxy bases) were investigated for their effects on urine volume and electrolyte excretion in male Wistar rats. All basic derivatives enhance urine volume and sodium excretion. The hydroxy bases show a stronger antikaliuretic effect than the unhydroxylated derivatives. In the group of hydroxy bases the influence of the substitution grade of nitrogen and the role of the pK_a values are discussed in order to establish possible structure–activity relationships regarding the antikaliuretic effect.

KEY WORDS: triamterene, pharmacodynamics; triamterene derivatives, structure–activity relationships; triamterene derivatives, effect on urinary electrolytes; diuretics potassium-sparing.

INTRODUCTION

Triamterene (TA; 2,4,7-triamine-6-phenylpteridine) belongs to the group of potassium-sparing diuretics. In the therapy of edema and hypertension it is used mainly in combination with thiazides and loop diuretics. The combined administration of these natriuretic agents together with triamterene increases natriuresis and reduces potassium loss.

As triamterene is only poorly soluble in water (25 mg/liter) (1), the drug is not commercially available for parenteral application. The metabolites of triamterene, the phase I metabolite 4-hydroxy-TA (OH-TA) and the phase II metabolite TA-sulfuric acid ester (OH-TA ester), are also only poorly water soluble (2). Both have natriuretic activity but weaker K^+-sparing properties than triamterene, i.e., they still have pharmacological activity (3,4). As the pharmacological activity was still present in these *p*-substituted triamterene analogues, several ethers of hydroxytriamterene with basic, neutral, and acidic groups were synthesized with the aim to find better water-soluble compounds with natriuretic and potassium-sparing properties similar to those of triamterene itself. Most of these compounds indeed showed diuretic activities similar to or better than those of triamterene (5,6). Of the basic triamterene derivatives, 4-(3-dimethylamino-2-hydroxypropoxy)triamterene (H3) was the most promising compound (7).

In this paper possible structure–activity relationships among the basic triamterene derivatives are discussed. Derivatives with straight and branched side chains and with a hydroxyl group in the basic side chain were included in these studies (Figs. 1 and 2). The effects on urine volume and sodium and potassium excretion were investigated in a screening model for diuretics. By performing dose–response experiments the triamterene derivatives were tested regarding their dose-dependent diuretic effect. Further, the possible interrelationship between the K^+-sparing effect and the pK_a value is discussed.

MATERIALS AND METHODS

Materials

The compounds tested were kindly supplied by Röhm Pharma, Weiterstadt, GFR.

Instruments

Electrolytes were measured by flame photometry using the Electrolyt Automat FL 6 (Zeiss, Oberkochen, GFR).

Animals

For the experiments male Wistar rats weighing 130–170 g were used. The rats were kept in an air-conditioned and light–dark-controlled (12 hr:12 hr) animal unit at 22°C and a relative humidity of 50%. They received a standard laboratory diet (Altromin) and tap water ad libitum. Food was withdrawn 18 hr prior to the experiments, but the rats had unrestricted access to water during this time.

Pharmacodynamics

Dose–Response Experiments. The investigations were performed with different doses of B3 as an example of a basic derivative, H3 as an example of a hydroxybase, and triamterene. H3 and B3 were dissolved in 0.9% saline by subsequently adding small amounts of 0.1 *N* HCl. Triamterene was suspended in water containing 20% polyethylene

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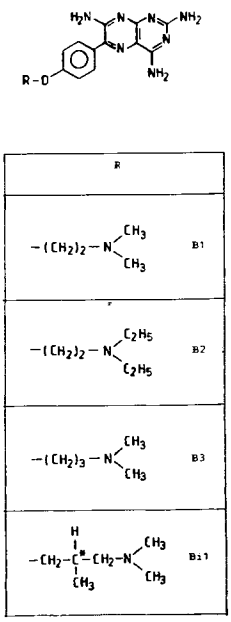

Fig. 1. Structural formulas of basic TA derivatives: 4-(2-dimethylaminoethoxy)triamterene (B1), 4-(2-diethylaminoethoxy)triamterene (B2), 4-(3-dimethylaminopropoxy)triamterene (B3), and 4-(3-dimethylamino-2-methylpropoxy)triamterene (Bi1).

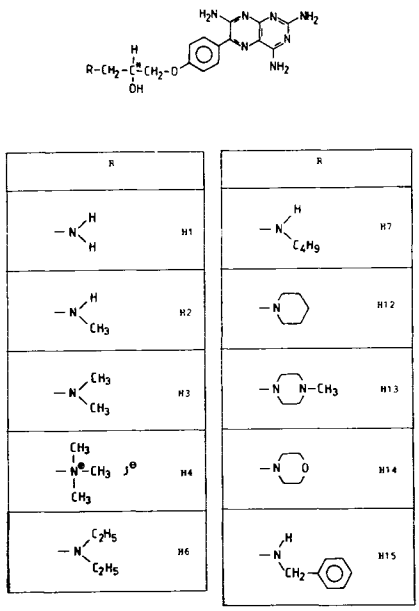

Fig. 2. Structural formulas of basic hydroxy derivatives: 4-(3-amino-2-hydroxypropoxy)triamterene (H1), 4-(3-methylamino-2-hydroxypropoxy)triamterene (H2), 4-(3-dimethylamino-2-hydroxypropoxy)triamterene (H3), 4-(3-trimethylammonium-2-hydroxypropoxy)triamterene-jodide (H4), 4-(3-diethylamino-2-hydroxypropoxy)triamterene (H6), 4-(3-butylamino-2-hydroxypropoxy)triamterene (H7), 4-(3-piperidino-2-hydroxypropoxy)-triamterene (H12), 4-(3-methylpiperazino)-2-hydroxypropoxy triamterene (H13), 4-(3-morpholino-2-hydroxypropoxy)triamterene (H14), and 4-(3-benzylamino-2-hydroxypropoxy)triamterene (H15).

glycol 400. For the evaluation of the dose–response curves 11 different doses between 0.01 and 100 μmol/kg body weight were applied to two animals each (8,16). Five animals served as controls, i.e., they received solvent only.

For standardization all rats received 20 ml/kg body weight saline by gavage. Shortly afterward the test compound or the solvent only was injected into one of the caudal veins under light ether anesthesia.

The rats were placed into individual metabolism cages without food and water. All experiments were started at 9 AM. The urine of each single rat was collected until 2.5 hr after injection and its volume measured. The sodium and potassium concentrations were analyzed by flame photometry, and the amounts excreted were calculated.

According to a method described previously (5) the coefficients of the following model function were fitted to the data on urinary Na^+ and K^+ excretion by nonlinear least-squares regression analysis using the NONLIN program (17) on a DEC 10 computer (18):

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

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 (= 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.

Screening Experiments. The substances were dissolved in saline by adding small amounts of 0.1 *N* HCl. Triamterene was suspended in water containing 20% polyethylene glycol (PEG) 400. The animals were randomly divided into groups of six rats each. The groups treated with diuretics were compared with a control group in each experiment. After having received saline by gavage for standardization, the test compound (verum group) or the solvent only (control group) was administered to the rats by intravenous injection. Control animals received intravenously water containing 20% PEG 400 in the experiments in which triamterene was included and saline in the other experiments. In each experiment only preparations of the same pH value and the same solvent were combined. The investigations were performed as described above, and the urine volume and sodium and potassium excretion were measured. The data obtained from the screening experiments are expressed as arithmetical

Table I. Fitted Coefficients of the Model Function Describing the Dose-Dependent Natriuretic (A) and Antikaliuretic (B) Effect of the Tested Substances

Compound	ED_{50} (μmol/kg)	E_0 (mmol/kg)	E_{max} (mmol/kg)	P
A				
H3	9.36	1.11	3.83	1.49
B3	5.53	0.73	4.45	0.98
TA	4.40	1.02	1.54	20.52
B				
H3	0.24	0.259	−0.203	1.74
B3	0.54	0.478	−0.332	1.16
TA	1.87	0.201	−0.146	1.17

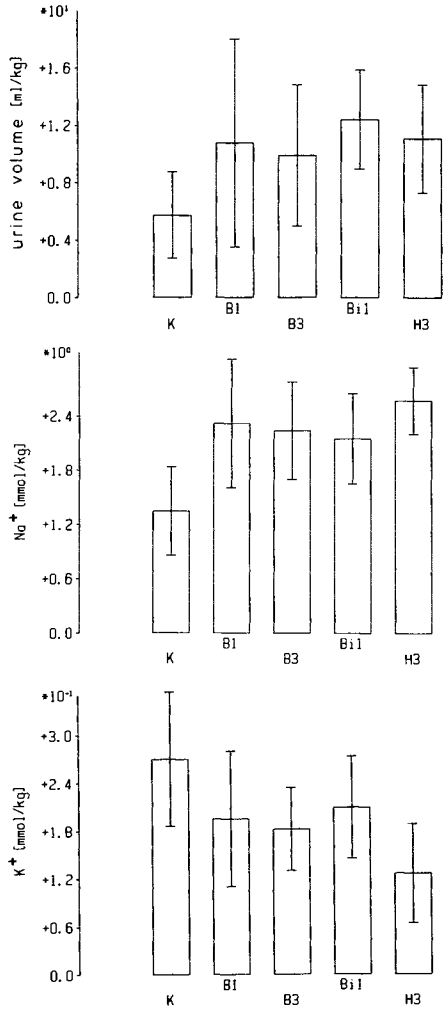

Fig. 3. Urine volume and sodium and potassium excretion ($\bar{X}$ + SD) after iv application of B1, B3, Bi1, and H3 (dose, 1 μmol/kg) during 2.5 hr (K, control).

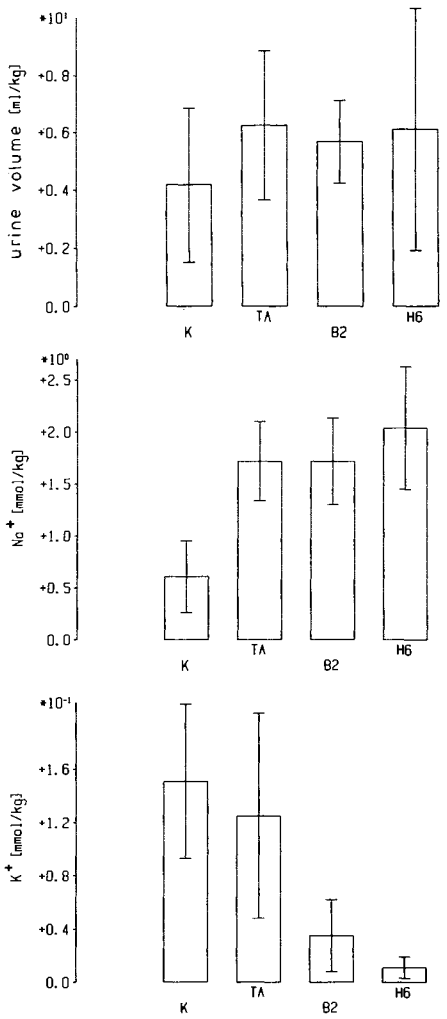

Fig. 4. Urine volume and sodium and potassium excretion ($\bar{X}$ + SD) after iv application of triamterene (TA), B2, and H6 (dose, 5 μmol/kg) during 2.5 hr (K, control).

means (N = 6) and standard deviation. Means and SD are recorded as histograms.

RESULTS

Dose–Response Experiments

To ensure that the effect is dose related, the dose–response curves of three of the investigated compounds were estimated, and the coefficients of the described model function were calculated. The fitted coefficients (potassium and sodium excretion) for the tested compounds are shown in Table I. The data show that both the natriuretic and the antikaliuretic effects are dose dependent for the investigated compounds (5,8,16,19). As all derivatives are rather similar, these results can be extrapolated to the whole group. Thus the prerequisite for the screening experiments can be looked at as fulfilled.

Screening Experiments

The results obtained from the screening experiments are shown in Figs. 3–7. These results were used to investigate semiquantitatively whether the intensity of the antikaliuretic effect depends on structural properties of the molecules.

All investigated basic derivatives enhance the urine volume and sodium excretion to nearly the same extent, compared to the control (K) (Fig. 3). Potassium excretion is lowered by all the compounds. Among these basic compounds with an additional hydroxyl group in the side chain, the alkanolamine derivative (hydroxy base) has the greatest potassium-retaining effect.

It can also be seen in Fig. 4 that the antikaliuretic effect of the hydroxy base (H6) is stronger than the effect of the unhydroxylated basic derivative (B2) or triamterene (TA).

Basic analogues with a hydroxyl group in the side chain were therefore studied regarding possible structure–activity relationships. In Fig. 5, the effects of three hydroxy bases (H2, H7, H15) are shown. The K^+-sparing effect decreases slightly from H2 (N-methyl derivative) over H7 (N-butyl derivative) to H15 (N-benzyl derivative). Furthermore, the activity of alkanolamines, whose side chains contain a heterocyclic ring, were tested (Fig. 6). Urine excretion is barely affected by these hydroxy bases (H12, H13, H14), whereas

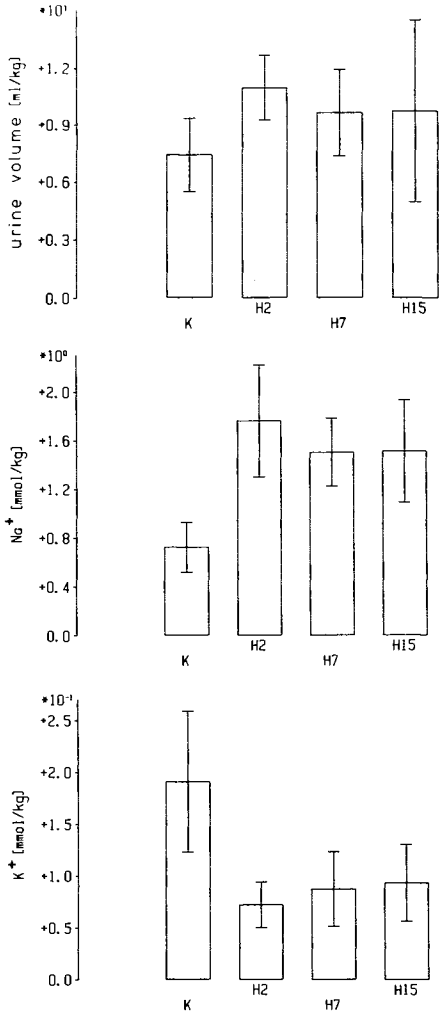

Fig. 5. Urine volume and Na^+ and K^+ excretion ($\bar{X}$ + SD) after iv application of H2, H7, and H15 (1 μmol/kg) during 2.5 hr (K, control).

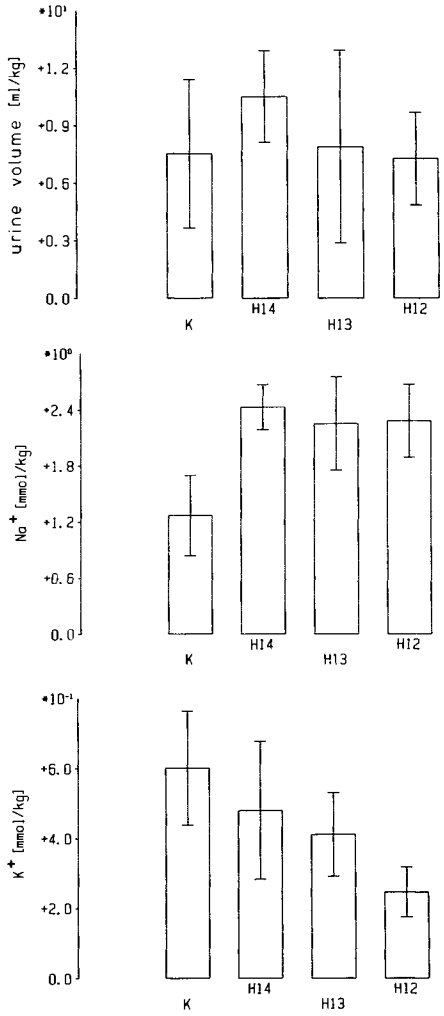

Fig. 6. Urine volume and Na^+ and K^+ excretion ($\bar{X}$ + SD) after application of H12, H13, and H14 (1 μmol/kg iv) during 2.5 hr (K, control).

the sodium excretion is strongly increased compared to the control (K). The potassium-retaining activity increases in the following sequence: morpholine < piperazine < piperidine derivative.

The influence of the substitution grade of the nitrogen in the ether side chain of the methylated hydroxy bases $-NH_2$ (H1), $-NH-$ (H2), $>N-$ (H3), and $>\overset{+}{N}<$ (H4) concerning electrolyte excretion was also investigated. The results of these studies are illustrated in Fig. 7. Urine volume and sodium excretion are enhanced by all hydroxy bases. Potassium retention increases in the following sequence: primary < tertiary < secondary amine. The quaternary ammonium compound shows the most clearly expressed K^+-sparing effect.

DISCUSSION

Other investigations from our group showed that neutral, acidic, and basic triamterene derivatives reveal a similar natriuretic potency, which thus seems to be independent of the structure and the charge of the compound. On the other hand, the antikaliuretic potency was different between the groups; the acidic compounds had the highest, and the basic substances had the lowest ED_{50} values (8,16,19). The E_{max} values (efficacy) of potassium excretion seem to be independent of the structures of the compounds. As a high correlation was found between the E_o and the E_{max} values for the potassium-sparing effect (5,8,16), 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_o).

On the basis of a nearly constant E_{max} of K^+ excretion for all tested substances, screening experiments can be performed, if the dose is the same in the single experiment and if the dose does not cause an effect near E_{max}. Under these conditions qualitative and even semiquantitative conclusions could be drawn regarding the antikaliuretic potency of the basic triamterene derivatives. After documenting the dose dependency of the effect, investigations were performed to evaluate tendencies in the group of basic derivatives that are due to certain structural properties, i.e., to describe structure–activity relationships regarding the antikaliuretic effect of the substances.

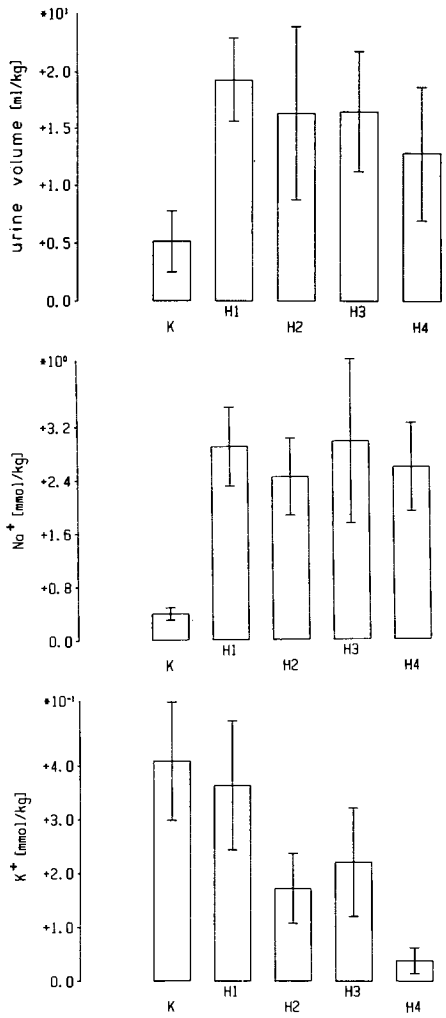

Fig. 7. Urine volume and Na^+ and K^+ excretion ($\bar{X}$ + SD) after application of H1, H2, H3, and H4 (2.5 μmol/kg iv) during 2.5 hr compared to control (K).

It was shown by Wolf (5) that basic ethers of 4-hydroxytriamterene exhibit stronger potassium-retaining properties than triamterene. Therefore, further derivatives with a basic side chain were investigated in order to test their diuretic effect. Prolongation of the side chain as well as branched side chains did not influence the natriuretic and antikaliuretic effect, whereas the introduction of a hydroxyl group in the β position to the nitrogen atom (hydroxy bases) led to a further increase in potassium retention.

In the case of hydroxy bases with differently substituted nitrogen in position 3 of the side chain, relationships between structure and electrolyte excretion were studied. While no differences could be detected regarding natriuresis, potassium retention decreases slightly from the *N*-methyl (H2) over the *N*-butyl (H7) to the *N*-benzyl derivative (H15).

The introduction of electronegative atoms (N, O) into the piperidine ring of 4-(3-piperidino-2-hydroxypropoxy)-triamterene (H12) seems to lower the potassium retaining effects. The piperidine derivative (H12) is more potent than the methylpiperazine derivative (H13), which is more potent than the morpholine derivative (H14). Regarding the substitution grade of nitrogen, the antikaliuretic activity increases in the following sequence: primary < tertiary < secondary amine. The quaternary ammonium derivative is the most active compound. From these results it can be concluded that the intensity of potassium retention depends on the basicity of the hydroxy base, i.e., on the pK_a value of the nitrogen atom in position 3 of the ether side chain.

Because of their physicochemical properties the experimental determination of the pK_a values of triamterene derivatives with conventional procedures is difficult (8,11). Taking into account that the molecules are identical except in their side chains, a relative approach can be made by comparing the pK_a values of the side-chain amines. The pK_a values of these amines are given in the literature (2,11,12,20–22).

The supposed relationship between potassium retention caused by the hydroxy bases and their pK_a values can be summarized as follows: the higher the pK_a value, the higher is the protonated portion and the better is the antikaliuretic effect of the according hydroxy base. As the positive charge is permanent and independent of the pH value in the quaternary hydroxy base, this compound possesses the most highly expressed potassium-retaining activity.

However, the postulated dependence of the antikaliuretic effect on the pK_a value cannot be observed in all cases. The primary hydroxy base has a lower K^+-sparing activity than the corresponding tertiary compound, although it is more basic (11). Furthermore, the introduction of a hydroxyl group in the β position decreases the basicity compared to that of the unhydroxylated compound (11); however, the hydroxy bases are more potent than the unhydroxylated bases. Possibly, this increase in the potassium retention of the hydroxy bases is caused by a certain conformation of the side chain (12) or an additional binding site at the receptor (13).

Different pharmacokinetic properties do not seem to play an important role when comparing the effects of the basic derivatives in these animal experiments (8,14). Previous investigations showed that the different effects are not due to different elimination half-lives and also not to biotransformation, as triamterene derivatives with basic side chains are not metabolized to a significant extent (14,7).

The proposed hypothesis that the potassium-sparing effect is dependent on the portion of the positively charged form is confirmed by further dose–response experiments (8,16,19) with the already mentioned neutral, acidic, and basic triamterene derivatives. From these studies it can be concluded that highly potent antikaliuretic diuretics of the TA type must have a basic character, i.e., must be positively charged at the physiological pH value (9,10). Further triamterene derivatives with a basic side chain (e.g., amidine and guanidine derivatives) are under investigation (15).

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