Pharmaceutical Research, Vol. 4, No. 1, 1987

Report

Dissociation of the Natriuretic and Antikaliuretic Properties of Triamterene Derivatives by Dose–Response Experiments

Helmut Priewer,[2] Erwin Wolf,[2] Helga Kraft,[2] Heinrich Knauf,[3] and Ernst Mutschler[2,4]

Received August 25, 1985; accepted September 29, 1986

Several derivatives of triamterene were synthesized with the aim of obtaining physicochemical properties superior to those of triamterene. Their effects on electrolyte excretion were tested with dose–response curves in rats: a dissociation of ED_{50} values of Na^+ excretion from those of K^+ retention was found; while the ED_{50} values of natriuresis were structure independent, the ED_{50} values for potassium retention depended highly on the charge of the side chain of the triamterene derivatives. Acidic compounds displayed low and amines high K^+-retaining potencies. Hence we postulate that there are at least two sites of action of the tested compounds in the kidney. (i) The first is the Na^+ conductance. Its blockade is responsible for the reduction in the lumen-negative electrical potential difference; this is the main driving force for K^+ secretion. The affinity to the Na^+ conductance is not correlated with the basic/acidic properties of the compounds. (ii) The second site is the finite K^+ conductance of the luminal membrane of the distal tubule. The affinity of the drugs to this K^+ conductance depends strongly on the charge of the molecule. Only pteridine derivatives with a basic side chain, i.e., with a high pK_a value, block the membrane K^+ conductance and are therefore potent potassium-retaining drugs.

KEY WORDS: triamterene, derivatives; diuretics, pharmacodynamics; diuretics, natriuretic and antikaliuretic response; diuretics, site of action.

INTRODUCTION

Potassium-sparing drugs are widely used in combination with saluretics for minimizing the excessive potassium excretion caused by the administration of saluretics alone. The most commonly used K^+-retaining compounds are amiloride and triamterene. In investigations studying the metabolism of triamterene (1,2), the metabolites were found to retain the pharmacological profile of the parent drug (3). Both hydroxytriamterene and hydroxytriamterene sulfuric acid ester proved to increase natriuresis and to decrease kaliuresis in the rat (4,5). In consequence, ethers of hydroxytriamterene with different chemical structures and various functional groups in the side chain were synthesized (Fig. 1), and their diuretic activities were tested in dose–response experiments in rats. The data were fitted to a model function which had proved in previous studies to be adequate to describe the observed dose-dependent diuretic response (7).

EXPERIMENTAL

Materials

Triamterene and all triamterene ethers were synthesized and donated by Röhm Pharma, Weiterstadt, GFR; all other chemicals were provided by E. Merck, Darmstadt, GFR. H3 and B3 were dissolved in 0.9% saline by subsequently adding small amounts of 0.1 *N* HCl. S3 and Si4 were dissolved in 0.9% saline by adding small amounts of 0.1 *N* NaOH. TA, A2, U1, and E3 were suspended in water containing 20% PEG 400.

Animals

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

Determination of Pharmacological Effects

The animals were randomly divided into the treatment groups. Under light ether anesthesia all rats received 20 ml/kg 0.9% saline by gavage shortly before the injection of the test solution in one of the caudal veins. For the evalua-

[1] This work is part of the Ph.D. thesis of H. Priewer.

[2] Institute of Pharmacology, Department of Biochemistry, Pharmacy and Food Chemistry, University of Frankfurt, Theodor-Stern-Kai 7, 6000 Frankfurt/M 70, GFR.

[3] Department of Medicine, University of Freiburg, Hugstetter Straße 55, 7800 Freiburg, GFR.

[4] To whom correspondence should be addressed.

0724-8741/87/0200-0066$05.00/0

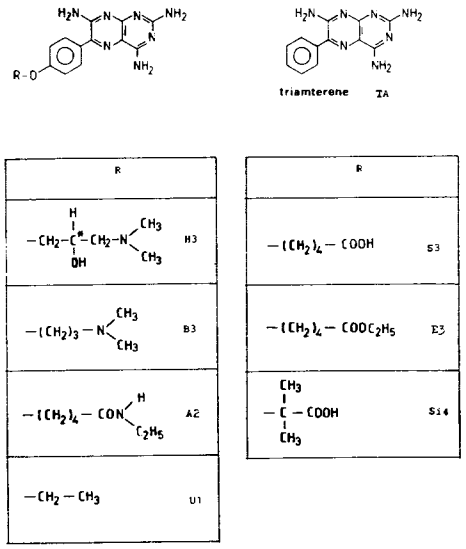

Fig. 1. Structures of the tested compounds and abbreviations used: TA, triamterene; H3, 4-(3-dimethylamino-2-hydroxypropoxy)triamterene; B3, 4-(3-dimethylaminopropoxy)triamterene; A2, ethyl amide of 4-(4-carboxybutoxy)triamterene; U1, 4-ethoxytriamterene; S3, 4-(4-carboxybutoxy)triamterene; E3, ethyl ester of 4-(4-carboxybutoxy)triamterene; Si4, 4-(1-carboxy-1-methylethoxy)triamterene.

tion of the dose–response curves 11 different doses between 0.01 and 250 μmol/kg body weight were applied to two animals each.

Five animals, serving as controls for each single dose–response curve, were treated with the corresponding solvent only. The rats were placed into individual metabolism cages without food and water. The experiments were carried out on different days but they were always started at about 9 AM. After a collection period of 2.5 hr the urine volumes were measured and the concentrations of Na^+ and K^+ were determined by flame photometry using the Electrolytautomat FL 6 (Zeiss, Oberkochen, GFR).

Statistics—Data Reduction

Equation (1) is derived from an equation given by Parker and Waud (6) to describe dose-dependent effects of drugs. To adapt this equation to experiments in whole animals, a minor modification is made, i.e., E_0 is introduced (7). This equation is used as a mathematical model to describe the measured data quantitatively in a reproducible fashion. The coefficients of this model function were fitted to the data of urinary Na^+ and K^+ excretion by nonlinear least-squares regression analysis using the program NONLIN (8) on a DEC 10 computer (9).

$$E = E_0 + E_{max} \frac{D^P}{D^P + ED^P_{50}} \quad (1)$$

D is the applied dose; E is the effect observed after the application of the dose D; E_0 is the basic excretion of ions without any drug applied ($D = 0$), i.e., the computer-fitted value for control animals; E_{max} is the maximal change in ion excretion that can be achieved by the test compound and represents the efficacy of the drug; and ED_{50} is the dose of the tested substance which produces a half-maximal effect. It is a measure of the potency of the drug; P is included in Eq. (1) to allow for variable slopes of the dose–response curves.

RESULTS

By performing dose–response experiments we were able to record the dose-dependent pharmacodynamic effects of various triamterene derivatives. Data reduction by fitting the parameters of the model function [Eq. (1)] to the experimental data provided quantitative measures of the observed pharmacological effects, thus facilitating the comparison of different compounds. Statistical criteria (signs and distribution of residuals) indicated that the chosen model function is suitable for this application. The fitted parameters E_0, E_{max}, ED_{50}, and P of sodium and potassium excretion for all tested compounds are shown in Tables I and II.

The doses which produce half of the maximal effect of the substances on sodium excretion were found to be of the same order of magnitude (3.5–10.3 μmol/kg) for all tested compounds; i.e., concerning sodium excretion these triamterene derivatives possess nearly the same potencies. In contrast, the potassium-retaining potencies varied widely. The ED_{50} values of acidic ethers of hydroxytriamterene (S3, Si4) are above 20 μmol/kg, and the ED_{50} values of neutral compounds (TA, A2, U1) lie in the range of 2 to 6 μmol/kg, whereas the values of basic derivatives (H3, B3) were found to be below 0.5 μmol/kg.

Due to the low water solubility and the small antikaliuretic effect of Si4, it was not possible to evaluate the complete dose–response curve of its potassium-retaining effect; up to the dose of 250 μmol/kg there was only a trend to K^+ retention, therefore the values for E_0 and ED_{50} were estimated.

The ester E3 is rapidly and almost completely metabolized, yielding the acid S3 (10), and therefore shows effects comparable to those of S3.

Figure 2 illustrates the relationships between the potencies of the compounds regarding sodium excretion and potassium retention. Compared to compounds with a neutral side chain (TA, U1, A2), it seems that a negatively charged side chain (S3, Si4) tends to diminish the potassium-sparing potency, while a positive charge in the same region of the

Table I. Fitted Coefficients of the Model Function Describing the Dose-Dependent Natriuretic Effects of the Tested Substances

Compound	ED_{50} (μmol/kg)	E_0 (mmol/kg)	E_{max} (mmol/kg)	P
H3	9.36	1.11	3.83	1.49
B3	5.53	0.726	4.45	0.976
TA	4.40	1.02	1.54	20.52
A2	3.50	1.18	4.09	1.46
U1	10.01	0.925	2.27	1.34
S3	5.86	1.37	3.32	17.19
E3	10.34	0.913	2.10	2.19
Si4	8.60	1.26	0.906	2.83

Table II. Fitted Coefficients of the Model Function Describing the Dose-Dependent Antikaliuretic Effects of the Tested Substances

Compound	ED_{50} (μmol/kg)	E_0 (mmol/kg)	E_{max} (mmol/kg)	P
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
A2	2.61	0.285	−0.260	1.41
U1	5.58	0.396	−0.367	2.23
S3	20.63	0.280	−0.220	28.21
E3	25.75	0.234	−0.222	1.07
Si4	>150	~0.3	—	—

molecule (H3, B3) seems to reinforce the potassium-retaining potency. However, the natriuretic potencies are the same for all compounds and are independent of the charge of the side chain.

While the control values (E_0) of sodium excretion were very similar to each other (0.7–1.4 mmol/kg), the values of the maximal effects (E_{max}) showed great variations (0.9–4.5 mmol/kg). Until now, no sure relationship could be found between these differences in the efficacies and the chemical structures or physicochemical properties of the compounds.

With regard to potassium excretion, E_0 as well as E_{max} varies less than the corresponding values for sodium excretion. Nevertheless, the E_{max} values differ approximately twofold (−0.146 to −0.367 mmol/kg). However, because of the very high correlation between E_{max} and E_0 (Fig. 3), all tested agents must be considered to possess the same efficacies.

DISCUSSION

All triamterene derivatives under investigation revealed a similar natriuretic potency, which thus seems to be independent of the structure and the charge of the introduced side chain. On the other hand, the ED_{50} values for the antikaliuretic effects were correlated to the charge of the side chain: the acidic compounds showed the highest and the basic substances exhibited the lowest ED_{50} values, the values of the neutral compounds ranging in between (Fig. 2).

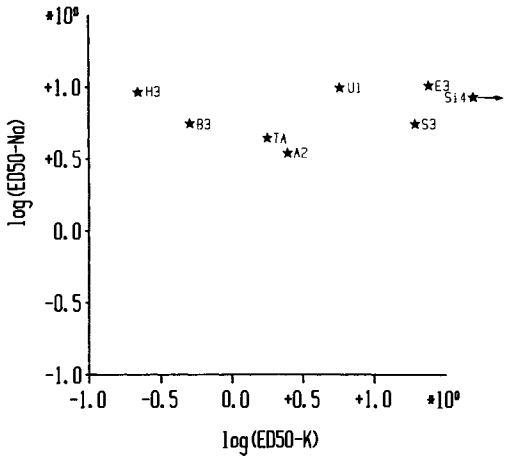

Fig. 2. Logarithms of the values calculated for ED_{50} of sodium excretion plotted against the logarithms of the values calculated for ED_{50} of potassium retention.

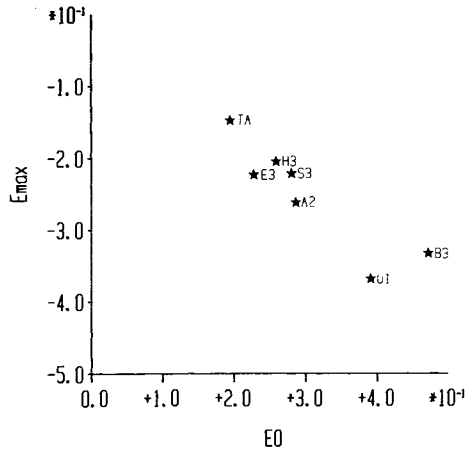

Fig. 3. Values calculated for E_{max} of the tested compounds on potassium retention plotted against the corresponding values calculated for E_0 ($r = -0.90$).

The natriuretic efficacies were found to vary but cannot be readily correlated with structural properties (11).

The E_{max} values of potassium excretion, however, are independent of the structures of the compounds; the high correlation (Fig. 3) of the values of E_{max} with the values of E_0 for the potassium-sparing effect indicates that the efficacies of the substances are similar, although the calculated values for E_{max} differ. However, these deviations in the calculated E_{max} values are caused by variations of the K^+ excretion of the controls (E_0), which was previously described by Wolf (7). Ignoring this correlation of E_{max} with E_0 in diuretic experiments may lead to misinterpretations of the efficacies of potassium-sparing diuretics if data from several experiments with different E_0 values are compared.

The different pharmacodynamic effects of the substances on sodium and potassium excretion can be explained as follows: the natriuretic potencies of the substances are independent of their antikaliuretic potencies, which indicates that the substances have more than one site of action. This is consistent with the findings of Guignard and Peters (12) and Kau (13), who proposed two sites of action of triamterene: a "secretory potassium transport site" and a "reabsorptive sodium transport site." The possible sites of action of potassium-retaining drugs, derived from *in vitro* experiments (14–17), are the sodium conductance and the Na^+/H^+ antiport. The sodium conductance is mainly responsible for the electrical asymmetry of the tubular cell, i.e., for the lumen-negative electrical potential difference (PD). In the distal tubule this PD is the main driving force for K^+ secretion through K^+ channels in the luminal membrane (18). Blockade of the Na^+ conductance by a pteridine derivative thus results in a reduced reabsorption of Na^+ accompanied by a diminished K^+ excretion.

It is still questionable whether the distal nephron possesses—in addition to the Na^+ conductance—a Na^+/H^+ antiport which also responds to triamterene and analogous drugs (15,17). As for the K^+ transport pathway in the distal

renal tubule, we are left only with the PD-driven K^+ secretion through the K^+ channels in the luminal membrane. An explanation for the dual effects of the different pteridine derivatives may be derived from their affinity to the membrane K^+ conductance, which is governed by the pK_a value of the agent. In the salivary duct epithelium (19), which closely resembles the distal nephron with respect to ion transport properties, the finite K^+ conductance was blocked only by amiloride (pK_a = 8.7) (20), and not by triamterene (pK_a = 6.2) (21), resulting in differing relative inhibitions of K^+ secretion. Thus, amiloride was the strongest K^+-retaining drug. In this context, it has to be kept in mind that the K^+ conductance is always smaller than the Na^+ conductance and it is less sensitive to drugs. Among triamterene derivatives the K^+ conductance is associated only with compounds carrying a basic side chain, i.e., with a high pK_a value (22).

The differences in the K^+-retaining potencies of the agents under investigation are considered to be due to their different affinities to the Na^+ and K^+ conductance of the distal tubule; a negative charge in the side chain (acidic derivatives) reduces and a positive charge (basic derivatives) increases the affinity to the K^+ conductance. This points to negative charges in the K^+ channels.

By performing dose–response experiments in the rat (*in vivo*), it was possible to show different sites of action of derivatives of triamterene by setting up structure–activity relationships. The triamterene part of the ethers is proposed to interact with the function of two ion transport systems in the kidney: the sodium conductance and the potassium conductance. The affinity of the agents to the Na^+ conductance (influencing the driving force for K^+ secretion) is less dependent on the charge of the side chain, while the affinity to the K^+ conductance, which regulates the secretion of K^+, is strongly dependent on the charge of the molecules. The described structure-dependent differentiation between the two effects of the compounds on electrolyte excretion may lead to the evaluation of more selective diuretics with reduced side effects.

REFERENCES

1. K. Lehmann. *Arzneim.-Forsch./Drug Res.* **15**:812–816 (1965).
2. B. Grebian, H. E. Geissler, and E. Mutschler. *Arzneim.-Forsch./Drug Res.* **26**:2125–2129 (1976).
3. H. Knauf, E. Mutschler, K.-D. Völger, and U. Wais. *Arzneim.-Forsch./Drug Res.* **28**(II):1417–1420 (1978).
4. G. Leilich, H. Knauf, E. Mutschler, and K.-D. Völger. *Magnesium Bull.* **1**:8–13 (1980).
5. G. Vollmer, E. Mutschler, and K.-D. Völger. *Arzneim.-Forsch./Drug Res.* **31**(I):529–530 (1981).
6. R. B. Parker and D. R. Waud. *J. Pharmacol. Exp. Ther.* **177**:1–12 (1971).
7. E. Wolf. Ph.D. thesis, Department of Pharmacology, University of Frankfurt, Frankfurt, GFR, 1981.
8. C. Daniel, and F. S. Wood. *Fitting Equations to Data,* J. Wiley & Sons, New York, 1980.
9. DECUS Program Library. DEC System-10 Catalog, Program No. 10-258, Digital Equipment Corp., Maynard, Mass., 1977.
10. H. Priewer, H. Kraft, and E. Mutschler. *Arzneim.-Forsch./Drug Res.* **35**(II):1544–1547 (1985).
11. H. Priewer, H. Kraft, and E. Mutschler. *Arzneim.-Forsch./Drug Res.* **36**(I):213–215 (1986).
12. J. P. Guignard and G. Peters. *Eur. J. Pharmacol.* **10**(2):255–267 (1970).
13. S. T. Kau. Ph.D. thesis, Nashville, Tenn., 1975.
14. H. Knauf and G. Sachs. In J. Rosenthal and H. Knauf (eds.), *Diuretika,* Verlag Chemie, Weinheim–New York.
15. P. S. Aronson. *Am. J. Physiol.* **245**:F647–F681 (1983).
16. H. J. Kramer, M. Rörig, and K.-D. Völger. *Pharmacology* **23**:149–156 (1981).
17. P. Vigne, C. Frelin, E. J. Cragoe, and H. Lazdunski. *Mol. Pharmacol.* **12**:131–136 (1984).
18. G. Giebisch, G. Malnic, R. M. Klose, and E. E. Windhager. *Am. J. Physiol.* **211**:560–568 (1966).
19. H. Knauf, R. Lübcke, W. Kreutz, and G. Sachs. *Am. J. Physiol.* **242**:F132–F139 (1982).
20. H. Knauf and R.Lübcke. *Pflugers Arch.* **361**:55–60 (1975).
21. H. Knauf, U. Wais, R. Lübcke, and G. Albiez. *Eur. J. Clin. Invest.* **6**:43–50 (1976).
22. H. Priewer, H. Spahn, and E. Mutschler. *Pharm. Res.* **3**:102–107 (1986).