remain to be determined. Aside from ISN cardiovascular effects, our results suggest that 2-ISMN is a potential anti-aggregating agent whose therapeutic use should be considered.

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Evidence for an Acute Anti-magnesiuretic Effect of Triamterene Derivatives[3]

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Abstract: The acute natriuretic, antikaliuretic and antimagnesiuretic effects of two triamterene derivatives, carboxybutoxytriamterene ethyl amide and dimethylaminohydroxypropoxytriamterene (RPH 2823), are shown in male Wistar rats during urine collection periods of 1 to 2.5 h. In combination with furosemide both compounds reduce the potassium excretion that is caused by the loop diuretic. Furthermore, RPH 2823 strongly decreases the magnesiuresis after application of furosemide, and the ethyl amide derivative (25 μmol/kg) reduces the magnesium losses produced by 25 μmol/kg furosemide close to control values. The evaluation of dose-response curves gave further evidence for the hypothesis that the renal handling of K^+ and Mg^{2+} is coupled to some extent.

The potassium retaining diuretic triamterene is widely used in combination with more potent saluretics like thiazides or loop diuretics to prevent the potassium losses which are caused by these compounds. Besides producing pronounced kaliuresis, diuretic agents such as furosemide increase magnesium excretion (1, 2), which may be responsible for some of the side effects of loop diuretic therapy.

In addition to its antikaliuretic effect, triamterene possesses magnesium retaining properties in untreated and in saline-loaded rats (3, 4) and in normal subjects (5, 6). In contrast to its antikaliuretic effects, the antimagnesiuresis produced by triamterene is not observed immediately after dosing but appears several hours afterwards.

Synthesis and pharmacological testing of triamterene derivatives with electron rich substituents of the side chain revealed similar natriuretic effects and even increased antikaliuretic potencies compared with triamterene (7, 8). Screening experiments demonstrated an acute antimagnesiuretic effect of two of these compounds, carboxybutoxytriamterene ethyl amide and dimethylaminohydroxypropoxytriamterene (RPH 2823) (Fig. 1).

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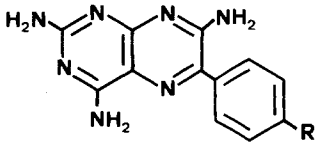

R = O-CH_2-CH(OH)-CH_2-$N(CH_3)_2$

RPH 2823

R = O-$(CH_2)_4$-C(=O)-NH-CH_2CH_3

Carboxybutoxytriamterene ethyl amide

Fig. 1 Structural formulas of dimethylaminohydroxypropoxytriamterene (RPH 2823), carboxybutoxytriamterene ethyl amide (ethylamide) and triamterene (R = H).

Materials and Methods

Materials

Carboxybutoxytriamterene ethyl amide, RPH 2823 and triamterene were kindly donated by Röhm Pharma, Weiterstadt, G.F.R. Furosemide was provided by Hoechst AG, Frankfurt, G.F.R. RPH 2823 was dissolved in saline by adding small amounts of 0.1 N HCl. Carboxybutoxytriamterene ethyl amide and triamterene were suspended in water containing 20% PEG 400. Furosemide was dissolved in saline.

Animals

Male Wistar rats weighing 130–170 g were used. They were kept in an air-conditioned and light-dark controlled (12 h : 12 h) animal unit with 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 h prior to the experiments, but the animals had unrestricted access to water.

Pharmacodynamics

The animals were randomly divided into groups of 6 rats. In each experiment the diuretic treated group was compared with a control that received the solvent only. For standardization and ensuring a sufficient diuresis in short experiments all animals received 20 ml/kg saline by gavage. Shortly afterwards the compound to be tested (or water/PEG 400 in the control groups) was injected in one of the caudal veins under light ether anesthesia. The rats were placed into individual metabolic cages without food or water. All experiments were started at approximately 9 a.m.

The urine of each rat was collected after 1 h (RPH 2823), 2.5 h (carboxybutoxytriamterene ethyl amide) and 1.5 h (combinations with furosemide) and the volume was measured. The sodium and potassium concentrations were analyzed by flame-photometry and the magnesium content by atomic absorption spectrometry using the Elektrolyt Automat FL 6 (Zeiss, Oberkochen, G.F.R.).

The arithmetic mean of each group was recorded in a histogram, and the standard deviations were specified. Differences between the study groups in magnesium excretion were assessed by the Kruskal-Wallis test (9, 10).

Dose Response Curves

For the evaluation of the dose response curves, 11 different doses between 0.01 and 50 µmol/kg were administered to 2 animals each. After a collection period of 2.5 h the urine volumes and the concentrations of Na^+, K^+ and Mg^{2+} were measured. The ED_{50} values were estimated by nonlinear regression analysis using the NONLIN computer program (11).

Results

Fig. 2 shows the sodium, potassium and magnesium excretion over 1 h after intravenous application of 6 µmol/kg RPH 2823. As shown earlier (7), RPH 2823 has natriuretic and pronounced antikaliuretic properties, but in addition the present experiment demonstrates the magnesium retaining effect of RPH 2823 ($p<0.1$). If 2.5 and 5 µmol/kg RPH 2823 are given together with 25 µmol/kg furosemide, the natriuresis which is considerably increased by the loop diuretic can be slightly raised, but RPH 2823 is able to reduce the intense potassium excretion produced by furosemide in a dose dependent manner to less than control values (Fig. 3). The urinary magnesium losses caused by furosemide are also decreased by RPH 2823 in a dose dependent way ($p<0.1$).

Fig. 4 shows the urinary electrolyte excretion over 2.5 h after i.v. application of 25 µmol/kg carboxybutoxytriam-

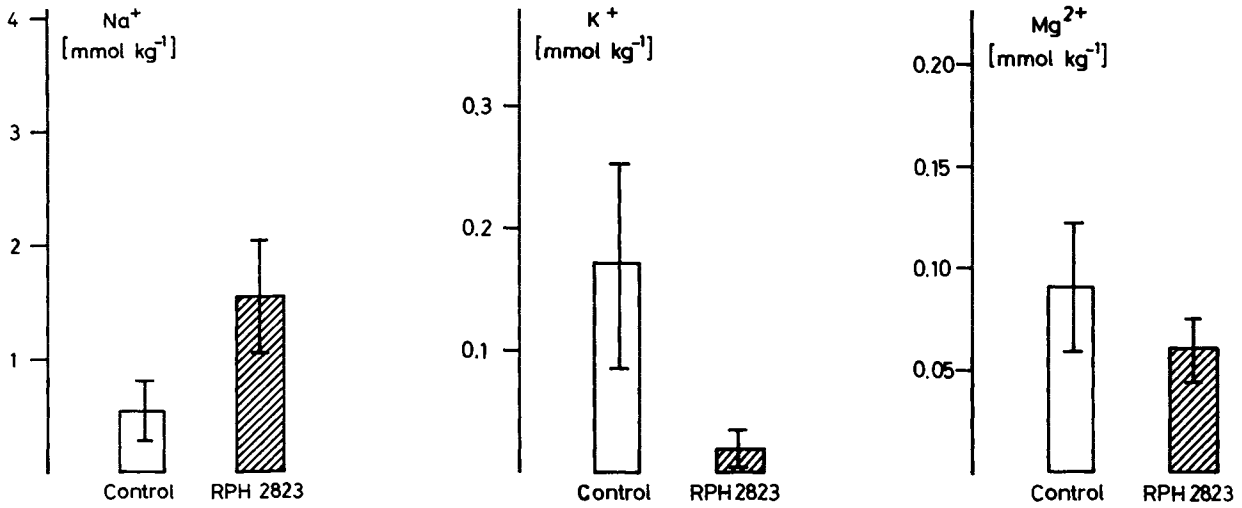

Fig. 2 Sodium, potassium, and magnesium excretion during 1 h after i.v. application of 6 µmol/kg RPH 2823.

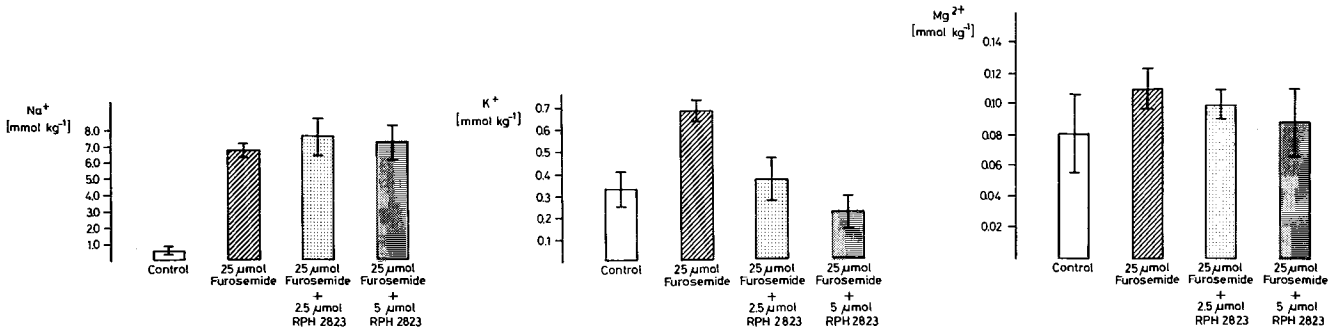

Fig. 3 Sodium, potassium, and magnesium excretion during 1.5 h after i.v. injection of 25 µmol/kg furosemide and combinations of 25 µmol/kg furosemide with 2.5 and 5 µmol/kg RPH 2823.

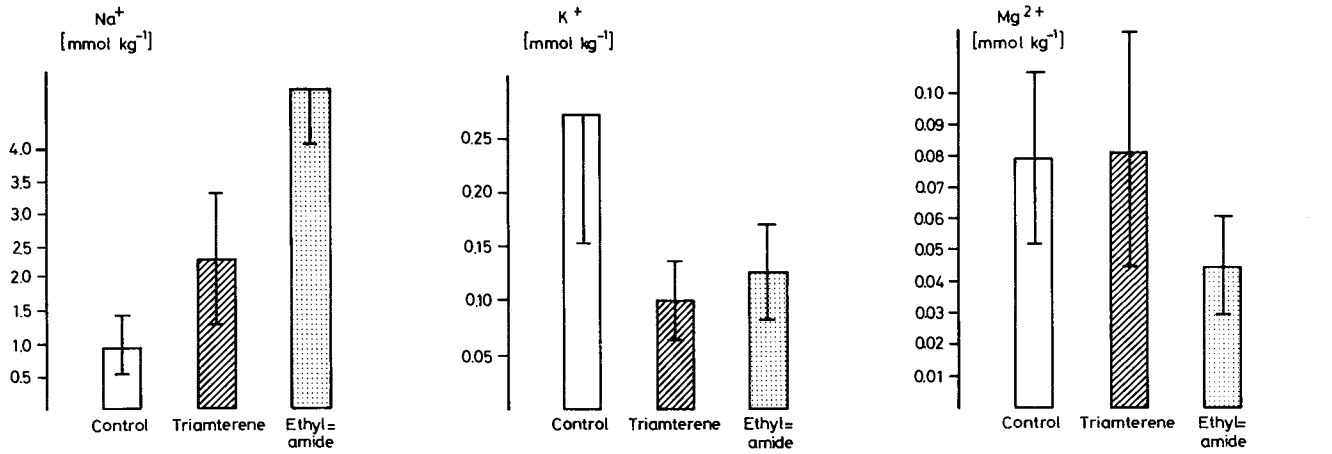

Fig. 4 Sodium, potassium, and magnesium excretion during 2.5 h after i.v. application of 25 µmol/kg triamterene and carboxybutoxytriamterene ethyl amide.

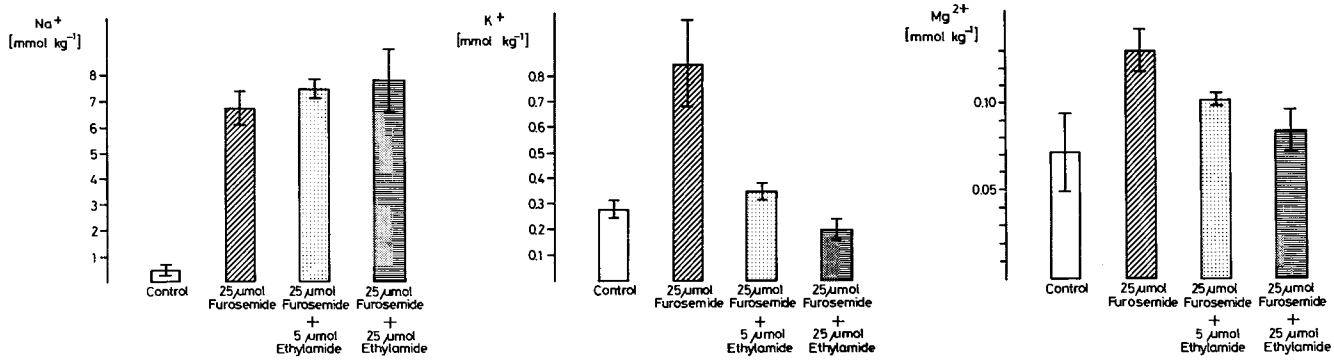

Fig. 5 Sodium, potassium, and magnesium excretion during 1.5 h after i.v. injection of 25 µmol/kg furosemide and combinations of 25 µmol/kg furosemide with 5 and 25 µmol/kg carboxybutoxytriamterene ethyl amide.

terene ethyl amide and triamterene, respectively. As already reported (8), sodium excretion is increased to a greater degree by the amide derivative than by triamterene. Both compounds exhibit a similar antikaliuretic effect. In contrast to triamterene, the amide reduces magnesium excretion markedly in this collecting period ($p<0.05$). If two different doses of the amide (5 and 25 µmol/kg) are combined with 25 µmol/kg furosemide (Fig. 5), there is a slight dose dependent increment in natriuresis. The high potassium excretion caused by furosemide is reduced in a dose dependent fashion to less than control values. 25 but not 5 µmol/kg of the amide prevent the magnesium losses produced by furosemide ($p<0.0005$).

Table I shows the ED_{50} values of the dose response curves calculated for excretion of urine, sodium, potassium and magnesium over 2.5 h after i.v. administration of RPH 2823 and the

amide. The values for urine volume and Na^+ excretion are comparable for both triamterene derivatives. The ED_{50} for K^+ and Mg^{2+} excretion differ by a factor of ten between the two compounds, but are similar within each substance.

Table. I. ED_{50} values of the dose response curves, calculated for the excretion of urine volume, sodium, potassium and magnesium during a 2.5 h collection period after i.v. application

Substances	ED_{50}	(μmol/kg)		
	urine volume	Na^+	K^+	Mg^{2+}
RPH 2823	5.81	9.36	0.24	0.40
Carboxybutoxy-triamterene ethyl amide	2.21	3.50	2.61	3.68

Discussion

To reveal the acute effects on magnesium excretion, the triamterene derivatives were tested in short experiments of 1 to 2.5 h (Triamterene exhibits its magnesium retaining property only several hours after dosing). In addition, short-acting loop diuretics like furosemide produce maximum elektrolyte excretion during the first and second hour after application.

The results presented in this paper indicate that carboxybutoxytriamterene ethyl amide and RPH 2823 are able to reduce magnesium excretion acutely in rats. The pronounced magnesiuresis that occurs after injection of furosemide can also be prevented. The amide depresses Mg^{2+} excretion almost to control values when given in the same molar dose as furosemide. RPH 2823 also reduces magnesiuresis caused by furosemide in a dose dependent way. For the proper interpretation of these results, it should be considered that RPH 2823 has a higher potency but a lower efficacy than carboxybutoxytriamterene ethyl amide concerning the magnesium retaining effect (12).

The data in Table I suggest a connection between the potassium and the magnesium retaining properties of the two compounds. Although RPH 2823 has a higher potency, in either case the ED_{50} values for the excretion of these two electrolytes are similar. These data support the observation (4, 15) that renal handling of K^+ and Mg^{2+} is coupled to some extent.

We have synthesized and tested a number of triamterene derivatives, i.e. carboxylic acids, amines, alcohols and amides. The carboxylic acids and the amines with no further functional group in the side chain had no acute magnesium retaining properties (12, 13, 14). An acute magnesium sparing effect has been demonstrated only with the compounds presented in this paper, i.e. the base with an additional -OH group and the amide, both having a N and an O atom. To further evaluate structure-activity relationships, we have tested hydroxyethoxytriamterene, a primary alcohol. Initial results indicate that it also possesses acute antimagnesiuretic properties, suggesting that a non-ionized O atom in the side chain may be required for the effect on magnesium excretion. This assertion will be the subject of further investigations.

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