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Torasemide, but not frusemide, increases intracellular cAMP and cGMP content in the aorta of the renal hypertensive rat

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Abstract—Repeated oral administration of the novel loop diuretic torasemide (3 mg kg^{-1}) and frusemide (30 mg kg^{-1}) for 7 days, elicited a significant fall in the systolic blood pressure in the one-kidney, one-clip Goldblatt renal hypertensive rat (RHR). The hypotensive action was greater in the torasemide group than in the frusemide group. Furthermore torasemide increased intracellular cAMP and cGMP content in aorta of RHR. Frusemide caused no effect. It is hypothesized that the increase in adenosine- or guanosine-nucleotides is involved in the antihypertensive action of torasemide, but not in that of frusemide.

Torasemide is a novel loop diuretic. Its chemical structure (1-isopropyl-3-{[4-(3-methyl-phenylamino)pyridine]-3-sulphonyl} urea) is not closely related to those of the other loop diuretics, such as furosemide. Torasemide exhibited a strong and long-lasting diuretic action when compared with frusemide in man (Lesne 1988; Lupinacci & Puschett 1988) and normal animals (Uchida et al 1990). The pharmacological profile of torasemide shows less kaliuresis in comparison with frusemide at doses which give equivalent natriuresis and aquaresis (Ghys et al 1985; Delarge 1988).

Torasemide produces an antihypertensive action at a lower dose than that which produces the diuretic action in man (Spannbrucker et al 1988). Thus, torasemide possesses an antihypertensive action independent of its diuretic one. However, little is known about the hypotensive mechanisms other than diuretic action. This study was designed to examine changes in the intracellular content of cyclic nucleotides (cAMP and cGMP) as a possible indication of a hypotensive mechanism of torasemide.

Materials and methods

Materials. Stock solutions of all drugs were prepared daily. Torasemide (Boeringer Mannheim GmbH, Germany) and furosemide (Nakalai Tesque, Japan) were dissolved in 0·1 M Na_2CO_3/0·1 M HCl, and were diluted further with physiological saline solution.

Methods. Renal hypertensive rats (RHR) were produced according to the one-kidney one-clip method (Garcia et al 1985). Briefly, normal male Wistar rats (Keari Co. Ltd, Japan) (ca. 200 g) were anaesthetized with pentobarbitone sodium (50 mg kg^{-1} i.p.) and a laparotomy was performed along the midline. After constriction of the left renal artery, the contralateral kidney was removed. Six weeks after the operation, animals which showed a systolic blood pressure greater than 160 mm Hg were selected and used in this study.

Drugs were administered to animals by gavage daily for 7 days. Systolic blood pressure (SBP) in the tail artery was measured by an indirect tail cuff method using a programmed electro-plethysmograph (PS-200A; Riken Kaihatu, Japan) in conscious animals. The measurement of SBP was carried out before and 4 h after drug administration at 1, 3 and 7 days after the start of the experiment.

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Aortic samples were excised from a pentobarbitone-anaesthetized animal 30 min after the final determination of SBP, and immediately frozen in liquid nitrogen. Intracellular content of cAMP and cGMP was determined according to the method of Honma et al (1977).

Statistics. Results are presented as mean ± s.e.m. Statistical significance of the difference between mean values was evaluated by Student's *t*-test (cyclic nucleotides) and paired *t*-test (SBP), and $P < 0.05$ indicated a significant difference.

Results

The hypotensive action of repeated oral administration of torasemide (3 mg kg^{-1}) and frusemide (30 mg kg^{-1}) in the RHR are shown in Fig. 1.

Torasemide and frusemide caused little effect on SBP at 1 day. But at 3 and at 7 days, a significant decrease in SBP was observed in both drug groups. Torasemide elicited greater hypotensive action than frusemide (Δ 46 mm Hg vs Δ 28 mm Hg) at day 7.

The effects of repeated oral administration of torasemide (3 mg kg^{-1}) and frusemide (30 mg kg^{-1}) on the intracellular content of cAMP and of cGMP in RHRs are shown in Fig. 2. In the saline treated control group, intracellular contents of cAMP and of cGMP were 713 ± 56 and 3·6 ± 0·3 fmol (mg wet wt)$^{-1}$ of aorta, respectively.

No significant change in the intracellular content of cAMP (715 ± 74 fmol mg^{-1}) and of cGMP (4·0 ± 0·4 fmol mg^{-1}) was observed in the frusemide group. In contrast, torasemide elicited significant increases in the intracellular content of cAMP (939 ± 87 fmol mg^{-1}, $P < 0.05$) and cGMP (5·3 ± 0·4 fmol mg^{-1}, $P < 0.01$). Torasemide caused an approximately 30% increase in

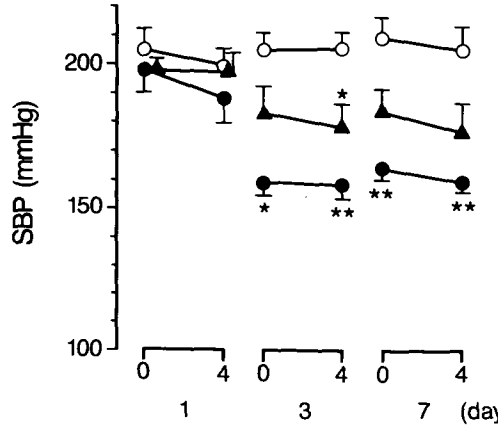

FIG. 1. Hypotensive effect of repeated oral administration of torasemide (●, 3 mg kg^{-1} day^{-1}, n = 8), frusemide (▲, 30 mg kg^{-1} day^{-1} n = 8) and vehicle (○, 10 mL kg^{-1} day^{-1}, n = 8). Systolic blood pressure (SBP) was determined before and 4 h after drug administration. Values are means ± s.e. ★, ★★ represents a significant difference from the value in the vehicle treated control group at $P < 0.05$ and 0·01, respectively.

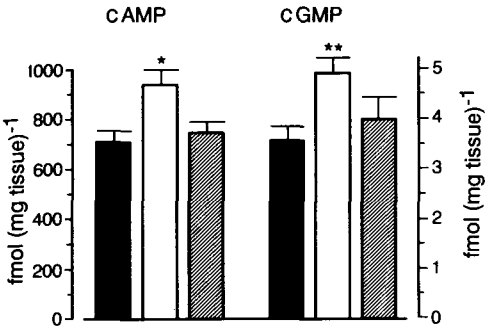

FIG. 2. Effect of repeated oral administration (7 days) of torasemide (□, 3 mg kg^{-1} day^{-1}, n = 8), frusemide (▩, 30 mg kg^{-1} day^{-1}, n = 8) and vehicle (■, 10 mL kg^{-1}, day^{-1}, n = 8) on intracellular cyclic nucleotides in the aorta of RHRs. Aortic samples were excised from a pentobarbitone-anaesthetized animal 30 min after the final determination of SBP. Values are means ± s.e. ★, ★★ represents a significant difference from the value in the vehicle treated control group at $P < 0.05$ and 0.01, respectively.

the intracellular content of cAMP and of cGMP within the 7 day period.

Discussion

The major antihypertensive mechanism of diuretics is related to a reduction in extracellular fluid and plasma volume (Shah et al 1978). Some investigators reported the possibility of dissociation between the antihypertensive and natriuretic effect of diuretics (Kraetz et al 1978). Long term administration of indapamide produces a hypotensive action at a dose which causes no significant alteration in body weight or plasma volume in man (Weidmann et al 1980). Torasemide shows an antihypertensive action at a non-diuretic dose in the hypertensive patient (Spannbrucker et al 1988). In contrast, the diuretic action of frusemide is the major mechanism of its hypotensive action.

We investigated hypotensive mechanisms of torasemide other than that due to the diuresis. Torasemide is 10 times more potent than frusemide in its diuretic action using either normotensive or renal hypertensive rats (Uchida et al 1991). There was a marked difference between the hypotensive action and the diuretic effect.

In the present study, frusemide caused no change in intracellular content of cAMP and of cGMP in rat aorta, while torasemide elicited a significant increase. It is known that an increase in intracellular cAMP and cGMP in the aorta causes vasodilation (Schoeffter et al 1987). Several phosphodiesterase inhibitors cause vasodilation by increasing the intracellular content of cAMP and of cGMP.

The mechanism of the torasemide-induced increase in intracellular cAMP and cGMP, however, remains unclear. A study to elucidate the mechanism of action is in progress.

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