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Acute blood pressure and urinary responses to single dose combinations of captopril and diuretics in conscious spontaneously hypertensive rats

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The present studies involved oral administration of captopril alone or in combination with individual diuretics in conscious, freely-moving spontaneously hypertensive rats (SHR). Both blood pressure (BP) and urinary fluid and electrolyte excretion were concomitantly measured. Captopril, 10 mg kg^{-1}, caused a decrease in BP (-18 ± 3 mm Hg) which was not significantly different from control (-10 ± 2 mm Hg). Diuretics alone also failed to alter BP. However the BP-lowering effect of captopril was readily potentiated by concomitant administration of a single oral dose of frusemide (10 and 30 mg kg^{-1}) in proportion to the magnitude of urinary loss. Hydrochlorothiazide at 1 and 3 mg kg^{-1} oral and metolazone at 0·3 and 1 mg kg^{-1} oral induced fluid loss similar to frusemide at 10 mg kg^{-1} and all produced comparable BP reduction in combination with captopril (25–32 mm Hg). Triamterene did not increase fluid excretion and was ineffective. In SHR with bilateral ureteral ligation, the synergistic effect of frusemide was abolished while that of hydrochlorothiazide still occurred with a delayed onset. It is concluded that concomitant measurement of BP and urinary excretion in conscious, freely-moving SHR is a useful technique for studying new antihypertensive drugs. Using this preparation it was found that enhancement of the acute hypotensive response to captopril with concomitant diuretic therapy occurs within 10 min and is primarily related to the fluid and Na loss, regardless of the diuretic agent used.

Diuretics are commonly used in the treatment of hypertension for their effective but limited antihypertensive activity as well as for prevention of secondary salt and fluid retention associated with the use of conventional antihypertensive drugs (Dollery 1977), permitting better control of arterial blood pressure (BP).

The intrinsic antihypertensive activity of angiotensin-converting enzyme inhibitors (ACEI) including captopril and enalapril is also limited in man and becomes enhanced only upon addition of diuretics such as hydrochlorothiazide and frusemide (Brunner et al 1980; Lijnen et al 1980; Ferguson et al 1982; MacGregor et al 1982; Weinberger 1982). Captopril and enalapril do not usually cause salt and fluid retention in man and animals (Antonaccio 1982; Ferguson et al 1982). Rather, combined use of ACEI and diuretics in antihypertensive therapy is primarily based on the supposition that the salt and fluid loss incurred by diuretics activates the renin-angiotensin system, which in turn assumes a greater role in the maintenance of BP and that as a consequence BP becomes more amenable to intervention by ACEI or other measures that interfere with the renin-angiotensin system (Ibsen et al 1978; MacGregor et al 1982).

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Spontaneously hypertensive rats (SHR) have been studied as a model of essential hypertension (Lais & Brody 1976). However, SHR are rather resistant to diuretic therapy; large doses of frusemide (30 mg kg^{-1}) and hydrochlorothiazide (20–100 mg kg^{-1}) cause little or no changes in BP (Inada et al 1977; Scriabine et al 1979; Chan et al 1982). In addition, SHR demonstrate only modest BP responses to acute administration of captopril (0·3–100 mg kg^{-1}) and the dose-response curve is rather flat (Laffan et al 1978; Sweet et al 1981). In two chronic studies, at least four days were required to effect a significant fall in BP in SHR treated with captopril 10 mg kg^{-1} orally (Muirhead et al 1978; Koike et al 1980).

In the present work, we studied the acute antihypertensive effect of captopril alone and with concomitant administration of single oral doses of different diuretics in freely moving conscious SHR. There was no previous volume depletion by multiple diuretic treatment (Miyamoto et al 1983). This preparation permits simultaneous BP measurements and urine collection so that the relationship between BP reduction and sodium and fluid loss can be evaluated. Through this study we attempt to establish a pharmacological basis on which an optimal combination of ACEI and diuretics is selected with regard to BP control.

METHODS

Male spontaneously hypertensive rats (SHR) (Taconic Farms, Germantown, NY), 16 weeks old, 300–350 g, were used. Test compounds were administered in 0·4% methylcellulose-saline suspension, 0·2 ml per 100 g weight, by gavage using a feeding needle. To measure BP and collect urine simultaneously, the animals were prepared one day before experiments according to the following procedure: The animals were anaesthetized with methohexitone (Brevital Sodium Lilly) 40–50 mg kg^{-1} i.p. The right carotid artery was catheterized with a PE 50 tubing filled with sodium heparin (300 unit ml^{-1}). The other end of the catheter was then passed under the skin and exteriorized on the back of the neck. A rat jacket (Alice King Chatham, Medical Arts, Los Angeles, CA) was used to support a rotating spring mount permitting the threading of the catheter through a spring to a swivel and on to a Statham pressure transducer on the day of experiments. In one study, 22 animals had a flank incision to ligate both ureters near the hilus. Animals were kept individually in metabolic cages, and were able to move freely. They had free access to food and water during recovery from surgery, but consumption was usually modest overnight.

On the following day, food and water were withheld during the experiments. The BP was allowed to stabilize and then recorded on a Grass polygraph for at least 30 min before oral administration of captopril, individual diuretics or combinations of both, to obtain a baseline. BP was then read at 30 min intervals for 5 h following drug administration. To ensure complete recovery, the urinary bladder was emptied by manual pressure at the beginning and end of the 5 h.

The sodium concentration of urine samples was determined with a flame photometer.

All data were expressed as mean ± standard error. Significant differences ($P < 0·05$) were determined by Duncan's multiple range test.

RESULTS

Blood pressure (BP) and renal responses to captopril and frusemide

The mean arterial blood pressure (MBP) in the SHR receiving vehicle remained relatively stable over 5 h (Fig. 1). Captopril 10 mg kg^{-1} caused a modest reduction in MBP 1–1·5 h after oral administration, but the changes were not statistically significant (Fig. 1; Table 1). This result was not influenced by concomitant administration of frusemide, 3 mg kg^{-1}. However, frusemide, at 10 and 30 mg kg^{-1}, potentiated the BP-lowering effect of captopril in a dose-related manner (Figs 1A and 2). Detectable falls in BP occurred as early as 10 min and reached maximum in less than 2 h. The potentiating effect of frusemide 30 mg kg^{-1} lasted at least 5 h. Frusemide alone in doses of 3, 10 and 30 mg kg^{-1} did not cause any significant alterations in MBP (Fig. 1B).

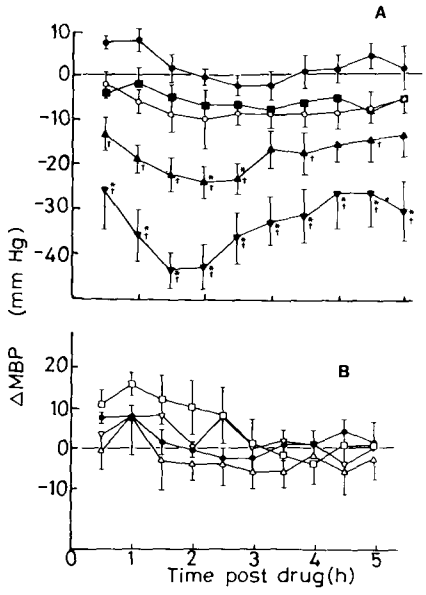

FIG. 1. Acute blood pressure effects of captopril and frusemide, alone or in combination, after oral administration in conscious SHR. Individual drugs were given at time zero as shown on the abscissa. On the ordinate are the changes in mean arterial blood pressure (Δ MBP). The baseline MBP was in average 176 mm Hg (range, 163–187 mm Hg) and there were no significant differences among the eight treatment groups. Each point represents mean ± standard error. (a) The time course of BP changes following MC vehicle and captopril alone or in combination with different doses of frusemide; (b) the time course of BP changes following MC vehicle or frusemide alone. ●—● vehicle (n = 10), ○—○ captopril (C) 10 mg kg^{-1} (n = 10), □—□ frusemide (F) 3 mg kg^{-1} (n = 6), △—△ F 10 mg kg^{-1} (n = 5), ▽—▽ F 30 mg kg^{-1} (n = 5), ■—■ C + F 3 mg kg^{-1} (n = 6), ▲—▲ C + F 10 mg kg^{-1} (n = 10), ▼—▼ C + F 30 mg kg^{-1} (n = 6). †$P < 0·05$ vs vehicle. *$P < 0·05$ vs captopril.

Captopril, 10 mg kg^{-1}, or frusemide, 3 mg kg^{-1}, had no detectable diuretic or natriuretic effects (Fig. 2). Frusemide, at 10 and 30 mg kg^{-1}, caused a dose-related increase in urine and sodium excretion. The magnitude of urinary responses to frusemide was not affected by the presence of captopril.

Blood pressure and renal responses to captopril in combination with other diuretics

Hydrochlorothiazide, 0·3 mg kg^{-1}, orally did not produce significant increases in urine and Na excretion and failed to potentiate the BP effect of

Table 1. Comparison of peak depressor responses, urine volume and sodium excretion due to captopril alone or in combination with hydrochlorothiazide, metolazone or triamterene in conscious SHR. Δ MBP, peak changes in mean arterial pressure in mm Hg over a 5 h interval postdrug; V, urine volume in ml/5 h per 100 g b wt; $U_{Na}V$, Na excretion in mequiv/5 h per 100 g b wt. †$P < 0{\cdot}05$, vs vehicle control; *$P < 0{\cdot}05$, vs captopril control (Duncan's multiple range test).

Treatment	n	Δ MBP	V	$U_{Na}V$
Vehicle	10	−10 ± 2	0·54 ± 0·07	0·05 ± 0·01
Captopril 10 mg kg^{-1} oral	10	−18 ± 3	0·52 ± 0·05	0·07 ± 0·01
Captopril + hydrochlorothiazide				
0·3 mg kg^{-1} oral	6	−16 ± 4	0·68 ± 0·06	0·10 ± 0·02
1·0 mg kg^{-1} oral	10	−28 ± 2†*	1·36 ± 0·11†*	0·25 ± 0·22†*
3·0 mg kg^{-1} oral	6	−27 ± 6†*	1·55 ± 0·16†*	0·30 ± 0·03†*
+Metolazone				
0·3 mg kg^{-1} oral	10	−32 ± 5†*	1·33 ± 0·20†*	0·19 ± 0·02†*
1·0 mg kg^{-1} oral	6	−32 ± 8†*	1·45 ± 0·18†*	0·31 ± 0·03†*
+ Triamterene				
1 mg kg^{-1} oral	10	−16 ± 3	0·59 ± 0·04	0·13 ± 0·01†*
3 mg kg^{-1} oral	5	−19 ± 4	0·58 ± 0·09	0·15 ± 0·03†*

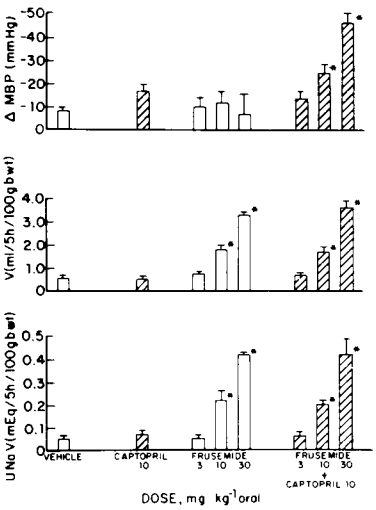

FIG. 2. The hypotensive, diuretic and natriuretic responses to captopril and frusemide, alone or in combination, after oral administration in conscious SHR. Data were obtained from same animals as presented in Fig. 1. Δ MBP represents peak BP drop over a 5 h interval in response to individual drugs. The urine volume (V) and sodium excretion ($U_{Na}V$) represent total urinary output over the same time interval per 100 g body wt. *$P < 0{\cdot}05$ vs vehicle or captopril alone.

captopril (Table 1). Triamterene, 1 and 3 mg kg^{-1} orally, increased Na excretion significantly but without accompanying diuresis and was also ineffective in rendering the animals more responsive to captopril. In comparison, hydrochlorothiazide at 1 and 3 mg kg^{-1}, and metolazone, at 0·3 and 1 mg kg^{-1}, produced marked diuresis similar in magnitude to that caused by frusemide 10 mg kg^{-1} (cf. Fig. 2). The treatments also resulted in similar natriuresis, with the exception that metalozone 0·3 mg kg^{-1} orally effected a lower sodium output. Concomitant administration of captopril, 10 mg kg^{-1}, with these drugs lowered BP to a similar extent and the fall was significantly greater than that caused by captopril alone. This augmentation by hydrochlorothiazide and metolazone of the antihypertensive responses to captopril showed a time course similar to that after frusemide (cf. Fig. 1).

None of the diuretics lowered BP significantly when administered alone (data not shown).

Blood pressure responses to captopril and diuretics in SHR with bilateral ureteral ligation

When the urinary fluid loss due to diuretics was prevented by bilateral ureteral ligation, a combination of captopril and frusemide (each 10 mg kg^{-1}) no longer produced changes in BP that were significantly different from captopril alone (Fig. 3). However, the antihypertensive effect of captopril in the animals treated with hydrochlorothiazide was still potentiated, but with a delayed onset. The fall in BP was significantly greater than that induced by captopril alone at 2·5, 3·5 and 5 h after dosing.

DISCUSSION

The acute antihypertensive response to a single oral dose of captopril is potentiated by concomitant administration of diuretic agents with a rapid onset in SHR. Thus it becomes evident that previous volume depletion with multiple diuretic treatment (Miyamoto et al 1983) is not necessary for this synergism to occur.

Intravenous studies in anaesthetized dogs by Imbs et al (1977) suggested that diuretics like frusemide cause an immediate increase in renin release through

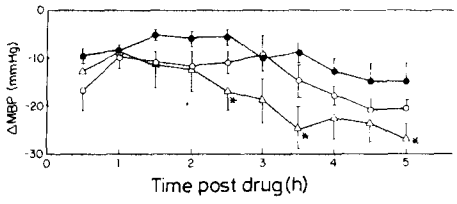

FIG. 3. Acute blood pressure effects of captopril (C) alone or in combination with frusemide (F) or hydrochlorothiazide (H) in conscious SHR with bilateral ureteral ligation. Drugs were given orally by gavage at time zero as shown on the abscissa. On the ordinate are the changes in mean arterial pressure (Δ MBP). The baseline MBP was 174 ± 7, 199 ± 4 and 186 ± mm Hg for C, C + H and C + F, respectively. Each point represents mean ± standard error. $^*P < 0{\cdot}05$, vs captopril control (Duncan's multiple range test). ●—● Captopril (C) 10 mg kg^{-1} oral (n = 6), ○—○ C + frusemide 10 mg kg^{-1} oral (n = 8), △—△ C + hydrochlorothiazide 1 mg kg^{-1} oral (n = 8). $^*P < 0{\cdot}05$, vs captopril control.

an action on the macula densa whereas diuretics like metolazone have a slow effect resulting from salt and water loss. It is not known whether a similar disparity exists in our experiments as a comparative study on the time course of renin release following oral administration of individual diuretics was not available. Nevertheless, diuresis usually occurs in ½–1 h after oral dosing with various diuretics in rats (Leuschner et al 1975; Chiu unpublished observation) and is presumably accompanied by stimulation of renin release. In the meantime, converting enzyme inhibition by captopril, as measured by suppression of pressor responses to angiotensin I, becomes evident as early as 20 min after oral administration in rats (Rubin et al 1978; Gross et al 1981). Under such circumstances, it is not unexpected that the BP-lowering effect of captopril would be readily unmasked through deactivation of the renin-angiotensin system which has assumed a greater role after fluid loss is effected by the diuretics. Miyamoto et al (1983) reported that the BP reduction following captopril administration was closely related to pretreatment plasma renin concentration in SHR undergoing 1-week diuretic therapy. Furthermore, there were no apparent differences in the time course of changes in BP in response to captopril and different diuretics in our study.

The acute antihypertensive effect of captopril was potentiated by frusemide in proportion to the magnitude of both Na and fluid loss. Furthermore, equipotent diuretic doses of frusemide, metolazone and hydrochlorothiazide produced similar BP lowering in combination with captopril. In contrast, triamterene, a K-sparing compound with modest natriuretic potency (Davies & Wilson 1975), caused a moderate increase in Na excretion but without diuresis and failed to augment the BP effect of captopril. Taken together, the results suggest that fluid loss plays an essential role in enhancing the acute antihypertensive action of captopril. Antonaccio et al (1979) demonstrated that increasing the dose of hydrochlorothiazide from 6 to 54 mg kg^{-1} orally did not further increase the hypotensive effect of captopril in renal hypertensive rats. This finding is expected as the doses used are supramaximal for diuretic effect in normal and hypertensive rats (Chiu unpublished observation).

When urinary loss was prevented by bilateral ureteral ligation the synergistic effect of frusemide and captopril was entirely abolished whereas some delayed effect was still evident with the use of hydrochlorothiazide 1 mg kg^{-1} orally. We have no satisfactory explanation for the latter finding. Hydrochlorothiazide in 0·3–0·7 × 10^{-3} M inhibits contractile responses of guinea-pig isolated aortic strips to angiotensin II and other vasoconstrictors (Davila & Davila 1981). Since a very low dose of the drug was used in our study, it is doubtful that a direct vascular effect was responsible for the BP effect. Chan et al (1982) also showed persistence of the synergistic antihypertensive effects with captopril (30 mg kg^{-1} oral) and hydrochlorothiazide (100 mg kg^{-1} oral) in SHR with bilateral ureteral ligation; the diuretic dosage they used was very high.

The urinary responses to frusemide and other diuretics were not altered by captopril in SHR. This is consistent with our recent finding that captopril did not affect the renal effects of frusemide in normal rats, unless the animals were previously under Na restriction (Chiu et al 1984).

It is well known that the antihypertensive effect of captopril is enhanced by preceding chronic treatment with a diuretic or a low sodium diet, or both, in man and animals in a normal or hypertensive state (Antonaccio 1982), but our results clearly indicate that combinations of captopril with diuretics that produced adequate fluid and salt loss are able to effect an immediate BP lowering after a single oral administration. Consequently, it is postulated that a compound with both ACE inhibitory and diuretic activities would be superior to either captopril or diuretics in the speed and effectiveness in control of hypertension. Concomitant measurement of BP and diuresis in conscious freely-moving SHR appears to be an appropriate preparation for testing this type of compound.

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