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Inhibitory effect of captopril on renal responses to frusemide in sodium-restricted rats

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Indomethacin (10 mg kg^{-1} orally) caused a moderate inhibition of the renal responses to frusemide (30 mg kg^{-1} orally) only in Na^+-deficient rats, suggesting that renal prostaglandins (PG) are necessary for optimal effects of frusemide during Na^+ restriction. Captopril (1, 3 and 10 mg kg^{-1} orally) also inhibited frusemide-induced diuresis and natriuresis in Na^+-deficient rats; the large effect of captopril at 10 mg kg^{-1} was accompanied by arterial hypotension. In normal rats, captopril did not disturb blood pressure or affect the renal effects of frusemide. By comparison, minoxidil (10 and 20 mg kg^{-1} orally) caused hypotension and reduced the natriuretic effects of frusemide in both normal and low-Na^+ states. Since circulating angiotensin II (AII) is a stimulus for PG synthesis during Na^+ restriction, it is suggested that captopril may impair the renal responses to frusemide through hormonal and haemodynamic changes resulting from inhibition of A II formation.

The renal prostaglandins (PG) constitute a major hormonal system in the kidney and play an important role in maintaining renal function during conditions of volume contraction, but exert little influence in the normovolaemic state (Epstein & Lifschitz 1980). We have previously reported that the potent natriuretic and diuretic actions of frusemide (furosemide), while unaffected in normal rats, was impaired in Na^+-restricted rats by cyclooxygenase inhibitors (Chiu & Long 1981) which suggested that the renal PG system is necessary for frusemide to produce optimal renal effects during Na^+ restriction.

Renal PGE_2 synthesis was markedly stimulated in rabbit (Stahl et al 1979) and in man (Rathaus et al 1981) during Na^+ restriction. The driving force for enhanced PG production is believed to be the increased angiotensin II concentration due to activation of the renin-angiotensin system resulting from Na^+ restriction or volume depletion (Dunn & Hood 1977; Satoh et al 1981). Captopril, a potent converting enzyme inhibitor, interrupts the renin-angiotensin system by blocking the formation of angiotensin II (AII) from the inactive angiotensin I (AI) (Antonaccio et al 1980). Attallah et al (1982) showed that in rabbits treated with captopril, PGE_2 synthesis in renal slices was substantially decreased. It is conceivable that this negative impact on the renal PG system by captopril would be more marked in the Na^+-restricted state and would possibly affect the natriuretic action of frusemide in a similar fashion to cyclooxygenase inhibitors (Chiu & Long 1981). Consequently the effects of captopril on the renal action of frusemide have been studied in normal and Na^+-deficient rats. However, in view of the hypotensive effects of captopril that may occur during an angiotensin-dependent state (Bengis et al 1978), the effects of minoxidil, a peripheral vasodilator (Haeusler & Gerold 1979), on the renal responses to frusemide were determined to evaluate non-specific blood pressure lowering as a factor.

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METHODS

Normal (93–193 g) and Na^+ deficient (95–208 g) male Charles River CD rats were fasted overnight before experiments. The Na^+ deficient rats had been kept on Sodium Deficient Diet (ICN Nutritional Chemicals, Cleveland, Ohio) for 13 days. The effectiveness of Na^+ restriction was reflected in the low urinary Na^+ excretion in these animals (0·0004 ± 0·0001 m equiv per 6 h per 100 g; cf. Chiu & Long 1981). The animals were housed individually in metabolic cages for continuous urine collection. All test drugs were prepared in 0·4% methylcellulose-0·9% NaCl suspension (volume of administration, 2·0 ml kg^{-1}). Two saline supplements were given 1 h (1 ml kg^{-1} s.c.) and 10 min (2 ml per rat, orally) before the oral or intravenous administration of frusemide, 30 mg kg^{-1}, which is the maximal diuretic dose in normal rats. Treatment with captopril, minoxidil, or indomethacin preceded furosemide by 60 min.

In a separate study, blood pressure effects with the same treatments were recorded for 3 h after each treatment in conscious rats. The animals underwent cannulation of the femoral artery under ether anaesthesia and were then kept in restrainers. At least 1 h was allowed for recuperation. The mean arterial pressure was measured with a Statham pressure transducer connected with a Grass polygraph.

The Na^+ concentration of urine samples was determined with a flame photometer (Radiometer); urine creatinine concentration was determined colorimetrically (Folin-Wu method). Urinary output of creatinine served as a crude index of glomerular filtration rate, on the assumption that plasma creatinine concentrations were stable over the 6 h experiment.

All data were expressed as mean ± standard error. Significant differences ($P < 0.05$) were determined by Student's *t*-test for paired comparisons; Duncan's multiple range test was used when more than two treatment groups were involved.

RESULTS

Effects of captopril pretreatment on urinary excretion and arterial pressure responses to oral frusemide.

Urinary excretion. The Na^+-deficient rats, compared with normal rats showed a diminished natriuretic response but a similar diuretic response to frusemide 30 mg kg^{-1} orally (Fig. 1). With captopril pretreatment (1, 3 and 10 mg kg^{-1} orally) the frusemide-induced increases in Na^+ and water excretion were unaffected in normal rats but were significantly ($P < 0.05$) inhibited in Na^+-deficient rats. The inhibition of urinary responses to frusemide caused by captopril at 10 mg kg^{-1} in the Na^+-deficient rats was accompanied by a significant fall in endogenous creatinine excretion (0·38 ± 0·11 vs 0·90 ± 0·08 mg per 6 h per 100 g in the control).

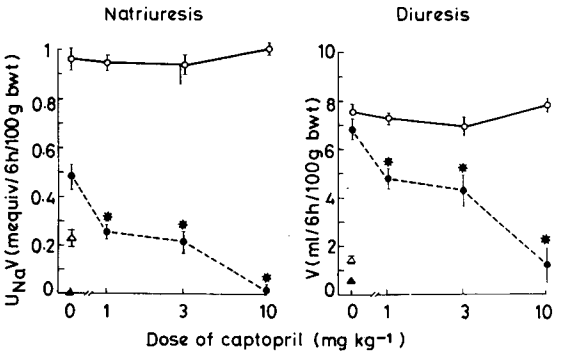

FIG. 1. Effect of captopril pretreatment on the natriuretic and diuretic responses to frusemide in normal and Na^+-deficient rats. The animals denoted by open or solid circles received frusemide, 30 mg kg^{-1} oral, 60 min after vehicle, or captopril, 1, 3 or 10 mg kg^{-1} oral. The open and closed triangles respectively represent 6 normal and Na^+ deficient-rats receiving neither frusemide nor captopril. *$P<0.05$, Duncan's multiple range test.

Table 1. Changes in mean arterial blood pressure in response to frusemide in conscious rats pretreated with captopril, minoxidil or indomethacin. The three test drugs were given by mouth 1 h before frusemide. Δ BP, changes in mean arterial pressure, represents an average of 3 h period following frusemide treatment (30 mg kg^{-1}). N.D., not done. *Effect of minoxidil alone; data from Chiu et al 1981. **$P<0.05$, vs vehicle control; Duncan's multiple range test.

Pretreatment + frusemide	Normal n	Normal Δ BP, mmHg	Low Na^+ n	Low Na^+ Δ BP, mmHg
Vehicle	6	1 ± 4	6	−1 ± 5
Captopril 1 mg kg^{-1} oral	—	N.D.	4	−4 ± 2
3	—	N.D.	4	−3 ± 6
10	6	−2 ± 2	6	−24 ± 4**
Minoxidil 5 mg kg^{-1} oral	6	−13 ± 3*	2	−12 ± 2
10	9	−20 ± 3*	3	−20 ± 4**
20	6	−24 ± 1**	6	−19 ± 2**
Indomethacin 10 mg kg^{-1} oral	—	N.D.	6	−6 ± 5

Arterial pressure (BP). In the Na^+-deficient rats, captopril (10 mg kg^{-1} orally), followed by frusemide (30 mg kg^{-1} orally), caused a significant fall in BP (Table 1). No significant changes in BP occurred in normal rats.

Effects of minoxidil pretreatment on urinary excretion and arterial pressure responses to oral frusemide.

Urinary excretion. In normal rats, minoxidil (10 and 20 mg kg^{-1} orally) inhibited frusemide-induced natriuresis ($P<0.05$) without significant changes in diuresis (Fig. 2). In Na^+-deficient rats, minoxidil (5, 10 and 20 mg kg^{-1} orally) diminished both natriuretic and diuretic responses to frusemide ($P < 0.05$). The creatinine excretion was not affected by minoxidil in any experiments.

Arterial pressure. In rats pretreated with minoxidil (5, 10 and 20 mg kg^{-1} orally), the fall in BP on subsequent administration of frusemide was similar in both normal and Na^+-deficient rats (Table 1).

Comparative effect of indomethacin and captopril on urinary responses to oral or intravenous frusemide in Na^+-deficient rats

Oral frusemide. In the normal rats neither indomethacin nor captopril, each at 10 mg kg^{-1} orally, altered the increases in water or Na^+ excretion after frusemide (data not shown). However, in the Na^+-deficient rats indomethacin reduced the

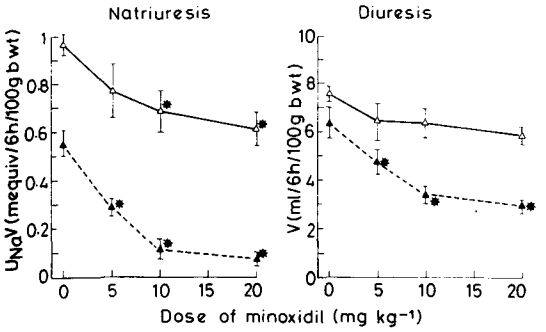

FIG. 2. Effect of minoxidil pretreatment on the natriuretic and diuretic responses to frusemide in normal and Na^+ deficient rats. All animals received frusemide, 30 mg kg^{-1} oral 60 min after vehicle, or minoxidil 5, 10 or 20 mg kg^{-1} oral. The group of normal rats receiving no minoxidil (zero dose) consisted of 8 rats. *$P<0.05$, Duncan's multiple range test.

frusemide-induced diuresis and natriuresis by 32 and 47% respectively (Table 2). By comparison, captopril inhibited the diuresis by 89% and the natriuresis by 99%, which were significantly greater than the indomethacin effects. BP was not significantly affected by indomethacin in the Na^+-deficient rats (Table 1).

Intravenous frusemide. Indomethacin diminished the frusemide-induced diuresis and natriuresis by 27 and 14%, respectively. The latter change, was not statistically significant ($P<0.10$). In comparison, captopril inhibited the water and Na^+ excretion by 52 and 70%, respectively. As in the oral study, captopril appeared to exert greater effects than indomethacin, but both drugs seemed to cause less inhibition of the urinary responses to frusemide given intravenously than when it was given orally.

DISCUSSION

The natriuretic and diuretic responses to frusemide were reduced with captopril pretreatment during Na^+ restriction but remained unaffected in a normal Na^+ state. It is likely that the hormonal or haemodynamic changes, or both, consequent to inhibition of AI converting enzyme by captopril (Bengis et al 1978; Johnston et al 1980) may influence the outcome of the renal action of frusemide, depending on the Na^+ status of the animals.

We have demonstrated that optimal renal responses to frusemide call for an intact renal PG system under conditions of Na^+ restriction (Chiu & Long 1981). Two different types of cyclooxygenase inhibitors, i.e. indomethacin and meclofenamic acid, were shown to inhibit the natriuretic and diuretic actions of frusemide in Na^+-restricted rats. Since A II and conditions (e.g. volume loss) associated with an increase in renin and angiotensin, result in an increase in renal release of PGE_2-like material (Dunn & Hood 1977), A II is thought to be an important stimulus of PGE_2 biosynthesis. If enhanced renal PGE_2 biosynthesis during Na^+ restriction mainly depends on A II (Stahl et al 1979), interruption of this tonic influence by inhibition of its converting enzyme would lead to a reduction in PGE_2 generation. In rabbits treated with captopril (0·5 mg kg^{-1} i.v.), PGE_2 synthesis was reduced by 47–85% in renal slices ex-vivo (Attallah et al 1982). Furthermore, i.v. administration of SQ 20, 881, a peptide-converting enzyme inhibitor, effectively inhibited the renal release of PGE-like material due to activation of the renin-angiotensin system by renal nerve stimulation or renal artery constriction in rabbits (Johns et al 1977). It is therefore suggested that captopril, like indomethacin, interferes with the renal effects of frusemide through inhibition of PGE_2 synthesis during Na^+ deprivation.

In contrast to the two studies mentioned above, both captopril and SQ 20, 881 were shown to selectively stimulate PGE_2 synthesis by rat isolated glomeruli, independent of angiotensin or bradykinin (Galler et al 1982). Moreover, Swartz et al (1980) and Moore et al (1981) reported that captopril (5–100 mg orally) increased the plasma concentra-

Table 2. Effects of indomethacin and captopril pretreatment on the diuretic and natriuretic responses to oral and intravenous frusemide in Na^+ deficient rats. V, urine volume in ml/6 h per 100 g; UNaV, Na excretion in mequiv h^{-1} per 100 g. *$P<0.05$, vs vehicle control; Duncan's multiple range test.

Pretreatment	Frusemide oral N	V	UNaV	Frusemide i.v. N	V	UNaV
Vehicle	5	6·4 ± 0·4	0·56 ± 0·05	6	5·0 ± 0·2	0·39 ± 0·02
Indomethacin 10 mg kg^{-1} oral	5	4·4 ± 0·4*	0·30 ± 0·05*	6	3·8 ± 0·4*	0·33 ± 0·04
Captopril 10 mg kg^{-1} oral	5	0·7 ± 0·3*	0·004 ± 0·002*	6	2·4 ± 0·3*	0·12 ± 0·02*

tions of the 13,14-dihydro-15-keto metabolite of PGE_2 in normal and hypertensive subjects. But Lijnen et al (1981) observed that captopril actually reduced plasma PGE_2 concentrations in hypertensive patients, as measured by radioimmunoassay. In spontaneously hypertensive rats, transient increase in urinary PGE_2 excretion followed oral captopril 100 mg kg^{-1}, but not 30 mg kg^{-1} orally (Miyamoto et al 1981). Consequently, whatever stimulatory effect captopril has on PG biosynthesis, direct or through kinin accumulation, our results, and those of others (Johns et al 1977; Attallah et al 1982), are more consistent with the hypothesis that removal of the potent stimulatory influence of A II during Na^+ restriction by AI converting enzyme inhibitor has the predominant impact, resulting in diminished renal PG synthesis, and that the renal action of frusemide is impaired in such circumstances.

The prompt diuresis due to frusemide is accompanied by activation of the renin-angiotensin system (Peart 1978). The rats we used were moderately volume-expanded with saline (2·0% body wt). In normal Na^+ rats, changes in blood pressure and electrolyte excretion after frusemide were unaltered by captopril pretreatment. Therefore stimulation of renin release by frusemide, which probably occurs later, exerts no significant influence on its own acute renal effects.

The inhibitory effects of captopril and indomethacin appeared to be less when frusemide was given to the Na^+-deficient rats intravenously instead of orally. Therefore the possibility that either drug decreases the renal effects of frusemide partially through interfering with its gastrointestinal absorption as a result of Na^+ restriction cannot be ruled out. Alternatively, renal tubular secretion of frusemide (Data et al 1978) may become vulnerable to captopril and indomethacin during Na^+ restriction, resulting in a diminished amount of drug to reach its site of action.

Lines of evidence indicate that endogenous renal PG's can affect epithelial NaCl transport in the distal nephron (Stokes 1981). However, the circumstances under which PG's facilitate the action of frusemide on the thick ascending limb of Henle's loop (Burg et al 1973) during Na^+ deprivation are unclear. In comparison with frusemide, the natriuretic effect of MK-447, a newly described diuretic agent, is mainly dependent on renal PG's and is inhibited by indomethacin even in normal Na^+ rats (Scriabine et al 1979).

Renal handling of Na^+ excretion is susceptible to fluctuations in BP (Schrier & de Wardener 1971). When BP is lowered, enhanced proximal tubular reabsorption of NaCl could occur with or without reduction in glomerular filtration rate (GFR, estimated by measuring creatinine excretion in this study), leading to a decrease in the amount of electrolytes reaching the distal nephron. Frusemide inhibits the active Cl^- transport which is responsible for NaCl reabsorption in the thick ascending limb of Henle's loop (Burg et al 1973). Consequently a decrease in distal delivery tends to reduce the efficacy of frusemide. This is well illustrated by the use of minoxidil, which lowers BP and concomitantly impairs the renal responses to frusemide regardless of body Na^+ status. Minoxidil is not known to interfere with PG formation and has actually been shown to increase blood concentrations of PG-like activity during arterial hypotension in dogs (Haeusler & Gerold 1979). In the Na^+-deficient rats, captopril at lower doses (1 and 3 mg kg^{-1}) or indomethacin, diminished the natriuretic action of frusemide without simultaneous changes in BP. However, hypotensive responses with an accompanying fall in GFR occurred with captopril at 10 mg kg^{-1}. The resulting inhibition of frusemide effects was greater than could be accounted for by inhibition of renal PG synthesis, as evidenced by the results with indomethacin. Therefore it is likely that arterial hypotension due to captopril may contribute to the inhibition of the natriuretic action of frusemide.

In conclusion, captopril inhibits the renal responses to frusemide in the Na^+ restricted state but not in the normal Na^+ condition. Presumably, captopril, by virtue of converting enzyme inhibition, blocks the tonic influence of angiotensin II on renal PG synthesis, which appears to be a prerequisite for opimal renal effects of frusemide during Na^+ restriction. However, arterial hypotension due to higher doses of captopril also appears to contribute to impairment of frusemide-induced diuretic and natriuretic effects.

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