Br J Clin Pharmacol 1996; **42**: 721–727

Effect of captopril on prostacyclin and nitric oxide formation in healthy human subjects: Interaction with low dose acetylsalicylic acid

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1 Angiotensin converting enzyme inhibitors have been suggested to act in part by potentiating the stimulatory effect of bradykinin on endothelial prostacyclin and/or nitric oxide (NO) formation. This may give rise to interaction with cyclo-oxygenase inhibiting drugs like acetylsalicylic acid, which is most often used in low doses in patients with cardiovascular diseases.

2 We investigated the effects of captopril (2×25 mg day^{-1}), or ASA (1×100 mg day^{-1}), or the combination of both drugs for 7 days, on blood pressure, prostanoid and NO formation rates in a double-blind, double dummy, randomized crossover study in 13 healthy female subjects. The urinary metabolites of thromboxane A_2 (2,3-dinor-TXB_2) and prostacyclin (2,3-dinor-6-keto-$PGF_{1\alpha}$), and PGE_2 were measured by gas chromatography/tandem mass spectrometry in urine on days 1, 6 and 7 of each medication. NO formation was assessed using urinary NO_3^- and cyclic GMP as indicators.

3 Urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ excretion was not significantly changed by either captopril, ASA, or their combination. Urinary 2,3-dinor-TXB_2 excretion was inhibited by $>80\%$ by ASA alone or in combination with captopril (each $P<0.05$), but was not affected by captopril alone. Urinary PGE_2 excretion was not significantly changed by either of the treatments. Urinary NO_3^- and cyclic GMP excretion rates were not significantly changed by captopril, ASA, or their combination.

4 Blood pressure was slightly reduced by captopril. ASA had no effect on blood pressure when given alone, nor did it modulate the effect of captopril on blood pressure during co-administration. Angiotensin II/angiotensin I ratio (index of ACE activity) was significantly decreased by captopril alone or in combination with ASA, but was unaffected by ASA alone.

5 Captopril does not stimulate prostacyclin formation in healthy human subjects in a dose sufficient to substantially inhibit ACE activity. Co-administration of ASA significantly inhibits 2,3-dinor-TXB_2 excretion, but does not interfere with the blood pressure lowering effect of captopril in healthy human subjects.

Keywords angiotensin converting enzyme inhibitors endothelium cyclic GMP bradykinin

Introduction

Angiotensin converting enzyme (ACE) inhibitors are being increasingly used in cardiac failure, coronary artery disease, and hypertension [1]. The angiotensin converting enzyme not only converts angiotensin (A) I into the biologically active A II, but it also degrades bradykinin, a highly potent biologically active peptide, into inactive fragments (kininase II activity) [2]. Bradykinin is well known as a potent stimulator of

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endothelial prostacyclin and nitric oxide (NO) formation [3]. Stimulation of the endothelial formation of these vasodilator autacoids has been suggested to contribute to the pharmacological effects of ACE inhibitors [4, 5]. Stimulation of prostacyclin and nitric oxide formation might also account for the vasoprotective effects of the ACE inhibitors, e.g. by modulating platelet/endothelial cell interactions and thromboxane formation. Therefore, low dose acetylsalicylic acid (ASA), which selectively inhibits platelet thromboxane formation [6], might interefere with the cardiovascular effects of captopril. Stimulation of prostacyclin formation by captopril and other ACE inhibitors [7–9], or potentiation of the stimulatory effect of bradykinin in the presence of ACE inhibitors has been shown in different experimental settings [10, 11]. Moreover, indomethacin has been shown to attenuate the hypotensive effect of ACE inhibitors [12, 13], possibly by inhibiting cyclo-oxygenase activity and thus blocking the prostaglandin-mediated part of the effect of the ACE inhibitors. However, there are few reliable studies on the effects of captopril on prostanoid formation rates in man using GC-MS analyses of their respective, enzymatically formed, urinary metabolites, i.e. 2,3-dinor-6-keto-prostaglandin (PG) $F_{1\alpha}$ for prostacyclin, and 2,3-dinor-thromboxane (TX) B_2 for TXA_2 [14].

Experimental data in animal models also suggest a role for NO in mediating the haemodynamic effects of ACE inhibitors [15, 16], which is supported by the observation that bradykinin is also a potent stimulator of endothelial NO formation [3]. Nitric oxide (NO) is rapidly oxidized to NO_3^- *in vivo* [17], which is then excreted into the urine. We have previously shown that NO formation *in vivo* can be assessed using the urinary excretion rates of NO_3^- and the second messenger cyclic GMP as non-invasive indicators [18–21].

We investigated in the present study whether captopril stimulates basal prostacyclin and/or NO formation in healthy human subjects, and whether co-medication with low dose ASA interferes with the effects of captopril on prostanoid excretion rates or on blood pressure.

Methods

Study design

Thirteen healthy female subjects in the salt replete state with a mean age of (mean ± s.e.mean) 24.9 ± 0.5 (range 22–30) years, a mean weight of 67.9 ± 1.4 (44–87.4) kg, and a mean height of 170 ± 5 (158–179) cm were included in this study after they had given their written informed consent. The subjects were advised on a constant, though uncontrolled sodium intake. The study protocol had previously been approved by the local Ethics Committee. The subjects received, in a randomized, double-blind, double-dummy, crossover design, either 2 × 25 mg captopril and 1 × aspirin placebo, 2 × captopril placebo and 1 × 100 mg aspirin, or 2 × 25 mg captopril and 1 × 100 mg aspirin daily, for 7 days. The study periods were separated by wash-out periods of 2 weeks. One day before each medication began, and on the 6th and 7th day of each medication, the subjects collected 24 h urines. Urine volumes were noted, and 50 ml aliquots were stored at −20° C for analysis of prostaglandin metabolites and creatinine. On the first day of each medication, the subjects remained in the supine position for at least 60 min before and 120 min after first drug intake. Blood pressure and heart rates were recorded immediately before and 120 min after first drug intake, and on the 7th day of each medication, using a semi-automatic device (Boso digital II; Bosch und Sohn, Jungingen, Germany). At baseline, as well as on the 6th and 7th day of each medication, a venous plasma sample was drawn for the measurement of angiotensin I and angiotensin II levels. Blood samples were centrifuged at 4° C immediately, and plasma was stored at −80° C until analysis.

Biochemical analyses

Quantification of urinary 2,3-dinor-TXB_2 (the major urinary metabolite of TXA_2), 2,3-dinor-6-keto-$PGF_{1\alpha}$ (the major urinary metabolite of prostacyclin), and PGE_2 was performed by negative chemical ionization gas chromatography-tandem mass spectrometry (GC/MS/MS) on a triple stage quadrupole mass spectrometer TSQ 45 (Finnigan MAT, San José, CA, USA) as described elsewhere [22, 23]. Briefly, endogenous prostanoids and their corresponding tetradeuterated internal standards, which had been externally added to 50 ml aliquots of urine samples, were extracted from acidified urine samples (pH 3.0) by solid-phase extraction on octadecyl silica cartridges (J.T. Baker, Deventer, The Netherlands). After derivatization to their pentafluorobenzyl ester methoxyamine derivatives and separation by reversed-phase h.p.l.c. the analytes were converted to their trimethylsilyl ether derivatives. GC/MS/MS was performed by selected reaction monitoring of the characteristic daughter ions generated by collision-activated dissociation of the corresponding parent ions for endogenous prostanoids and their stable-isotope labeled analogues.

Urinary NO_3^- excretion was determined by gas chromatography (GC) as described elsewhere [18]. The detection limit for this method was 320 pmol ml^{-1} while the intra- and interassay coefficients of variation were 3.5%.

Cyclic GMP content was measured by radioimmunoassay using [^{125}I]-cGMP as a tracer and globulin precipitation. The detection limit of the assay was 160 fmol ml^{-1}.

Plasma and urinary creatinine was determined spectrophotometrically by the alkaline picric acid reaction with an automatic analyser (Beckman, Galway, Ireland), and the urinary excretion rates of prostanoid metabolites, NO_3^- and cyclic GMP were corrected by urinary creatinine concentration as described previously [18, 19, 22]. Urinary sodium was measured by flame photometry.

Plasma angiotensin I and angiotensin II concentrations were determined by commercially available

radioimmunoassays (Peninsula Lab., Belmont, CA, USA) using the respective [^{125}I]-labelled peptides as tracers. Detection limits of the assays were 130 pg ml^{-1} for angiotensin I and 19 pg ml^{-1} for angiotensin II.

Statistical analyses

All values are given as means ± s.e.mean in the text and in the figures. Statistical analyses of prostanoid and NO metabolite excretion rates were performed using repeated measures analysis of variance followed by Scheffé's *F*-test for multiple comparisons [24]. The effects of the treatments on blood pressure were tested for statistical significance using Student's *t*-test. The minimum level of statistically significant difference was considered to be $P < 0.05$.

Results

Baseline measurements

ANOVA demonstrated that there were no period effects, i.e. the baseline values of the haemodynamic and biochemical parameters were comparable for all three study periods.

Prostanoid excretion rates

Mean baseline urinary 2,3-dinor-6-keto-PGF$_{1\alpha}$ excretion in the three medication periods was 222.4 ± 17.7 pg mg^{-1} creatinine. It was not significantly changed by either captopril (day 7, +10.3 ± 13.3% [95% confidence interval (CI) +105.9, −85.4%]), or ASA alone (day 7, +14.2 ± 29.8% [95% CI +228.9, −200.5%]), or their combination (day 7, −17.6 ± 15.7% [95% CI +95.7, −130.9%]) (Figure 1a). Mean baseline urinary 2,3-dinor-TXB$_2$ excretion in the three medication periods was 359.0 ± 39.5 pg mg^{-1} creatinine. It was inhibited by >80% by ASA alone (day 7, −57.9 ± 8.0% [95% CI −0.1, −115.8%]) or in combination with captopril (day 7, −71.3 ± 12.6% [95% CI −1.7, −174.5%]; each $P < 0.05$), but was not affected by captopril alone (day 7, −6.4 ± 15.7% [95% CI +107.1, −119.9%]; P = NS) (Figure 1b). Urinary PGE$_2$ excretion (baseline: 237.7 ± 24.6 pg mg^{-1} creatinine) showed a large interindividual variability and was not significantly changed by either of the treatments (captopril, +1.4 ± 25.0% [95% CI +181.5, −178.6%]; ASA, +68.1 ± 39.9% [95% CI +356.1, −219.9%]; captopril + ASA, +49.6 ± 67.7% [95% CI +537.7, −438.5%]; each P = NS).

Index metabolites of systemic NO production

Urinary NO_3^- excretion was 191.5 ± 20.4 μmol mmol^{-1} creatinine at baseline. It was not significantly changed by captopril alone (day 7, −33.8 ± 22.8% [95% CI

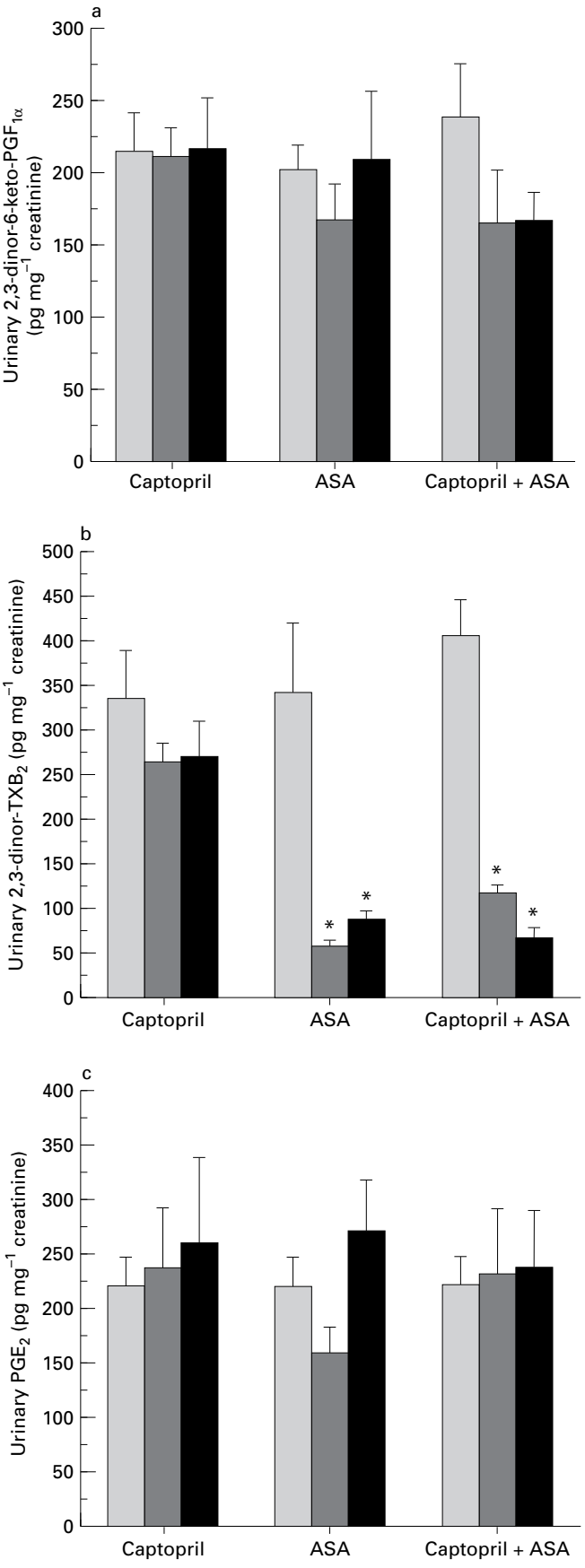

Figure 1 Effects of captopril (2 × 25 mg day^{-1}), ASA (1 × 100 mg day^{-1}), or their combination, on the urinary excretion rates of 2,3-dinor-6-keto-PGF$_{1\alpha}$ (a), 2,3-dinor-TXB$_2$ (b), and PGE$_2$ (c) before (basal ▨), and on days 6 (▩) and 7 (■) of each medication. Data are means ± s.e.mean of 13 healthy female subjects. *$P < 0.05$ *vs* day 1.

+124.4, −192.1%]) or in combination with ASA (day 7, −1.0±23.9% [95% CI +164.8, −166.9%]), nor by ASA alone (day 7, −0.8±22.5% [95% CI +155.1, −156.8%]) (Figure 2a). Urinary cyclic GMP excretion was 195.1±33.3 nmol mmol^{-1} creatinine at baseline. It was not significantly affected by captopril (day 7, −33.3±10.7% [95% CI +42.7, −110.3%]), ASA (day 7, −3.9±15.4% [95% CI +107.1, −114.8%]), or their combination (day 7, +27.2±36.5% [95% CI +290.4, −236.0%]) (Figure 2b).

Plasma A II/A I ratio

Plasma angiotensin II/angiotensin I (A II/A I) ratio was 0.310±0.083 at baseline. It was decreased to 0.052±0.010 ($P<0.05$) and to 0.056±0.010 ($P<0.05$) by captopril alone or in combination with ASA, respectively, but was unaffected by ASA alone. Plasma angiotensin I and angiotensin II levels and AII/A I ratios are given in Table 1.

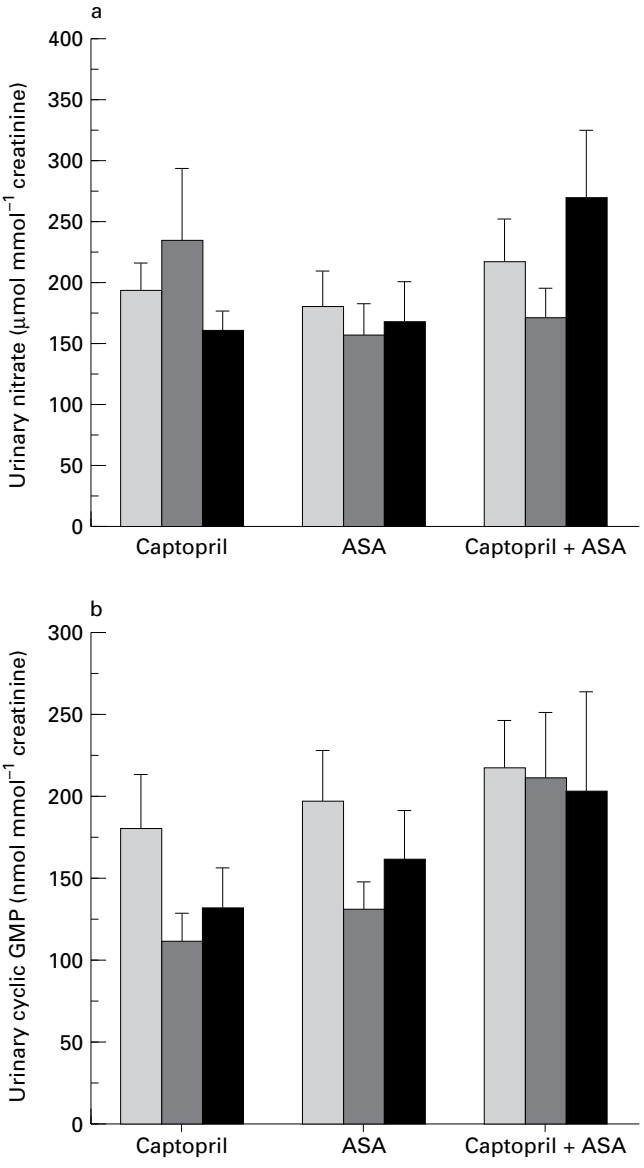

Figure 2 Effects of captopril (2×25 mg day^{-1}), ASA (1×100 mg day^{-1}), or their combination, on the urinary excretion rates of NO_3^- (a) and cyclic GMP (b) before (basal ▤), and on days 6 (▨) and 7 (■) of each medication. Data are means±s.e.mean of 13 healthy female subjects.

Effects on blood pressure

Blood pressure was 118.8±2.0/72.0±2.1 mm Hg (systolic/diastolic) at baseline. Systolic and diastolic blood pressure was slightly, but significantly reduced to 114.7±2.2/67.5±2.2 mm Hg at 120 min after captopril intake ($P<0.05$) and to 111.2±2.2/67.7±2.2 mm Hg after captopril +ASA intake ($P<0.05$), but remained unchanged 120 min after ASA intake (119.3±1.8/75.9±3.1 mm Hg). On day 7 of each medication, systolic blood pressure was not significantly different from baseline values after captopril, ASA, or their combination (118.3±2.9, 117.0±3.6, and 116.4±4.1 mm Hg, respectively), but diastolic blood pressure was significantly decreased after captopril and captopril + ASA (63.3±3.3 and 66.8±2.6 mm Hg, respectively), as compared with baseline (each $P<0.05$), while ASA alone had no effect on blood pressure on day 7 of drug intake (71.6±3.2 mm Hg). No significant changes in heart rates were observed with either of the treatments.

Discussion

Our present study suggests that the ACE inhibitor captopril does not measurably enhance basal prostacyclin or nitric oxide formation *in vivo* in healthy human subjects, in a dose sufficient to inhibit substantially plasma ACE activity. Moreover, there was no interaction between captopril and low dose acetylsalicylic acid on blood pressure or the formation of these vasodilator autacoids.

Captopril reduces the formation of angiotensin (A) II from A I by blocking the activity of the angiotensin converting enzyme (ACE); this can be reliably monitored using the plasma A II/ A I ratio, which correlates well with *in vitro* ACE activity measurements using synthetic substrates [25]. It is well accepted from *in vitro* pharmacological data that ACE inhibitors also potentiate the biological effects of bradykinin by inhibiting the kininase II activity of the same enzyme [2, 5, 7, 10, 26]. Bradykinin-induced vasodilatation in isolated perfused rabbit hearts was significantly enhanced by the ACE inhibitor ramiprilat; both co-administration of the cyclo-oxygenase inhibitor diclofenac or the NO synthase inhibitor nitro-L-arginine halved the vasodilator response to bradykinin [27].

However, data on the effects of ACE inhibitors on prostanoid formation *in vivo* are sparse and contradictory. Using radioimmunoassay analyses of non-enzymatically formed prostanoid metabolites, some authors reported that ACE inhibitors did not significantly increase the urinary excretion of 6-keto-$PGF_{1\alpha}$ in normal man [12, 28], although others did [29]. The cyclo-oxygenase inhibitor indomethacin reduced the hypotensive effects of ACE inhibitors in he majority of studies [13, 31, 32], although not in all [29, 30, 33].

Table 1 Plasma concentrations of angiotensin I, angiotensin II, plasma A II/A I ratios, creatinine clearances, and urinary sodium excretion in 13 healthy female subjects receiving 2×25 mg captopril/day 1×100 mg ASA/day, or their combination for 7 days.

	Captopril			*ASA*			*Captopril + ASA*		
Treatment	*Day 1*	*Day 6*	*Day 7*	*Day 1*	*Day 6*	*Day 7*	*Day 1*	*Day 6*	*Day 7*
AI	28.8	21.3	59.6	28.8	22.1	45.9	28.8	21.6	48.1
(pmol l^{-1})	±4.0	±2.3	±13.6	±4.0	±3.5	±11.2	±4.1	±4.6	±14.5
AII	7.3	1.5	2.1	7.3	5.3	4.8	7.3	1.3	1.5
(pmol l^{-1})	±1.6	±0.1	±0.4	±1.6	±0.7	±0.7	±1.5	±0.2	±0.3
AII/AI	0.310	0.088*	0.052*	0.310	0.348	0.309	0.310	0.085*	0.056*
	±0.083	±0.016	±0.010	±0.083	±0.092	±0.157	±0.083	±0.020	±0.010
CL_{CR}	219.4	236.5	258.4	219.4	158.0	269.4	219.4	229.7	231.1
(ml min^{-1})	±24.5	±58.9	±79.7	±24.5	±24.3	±48.5	±24.5	±60.4	±52.6
UVNa	158.0	171.4	172.2	157.7	149.9	151.2	160.6	167.7	174.0
(mmol 24 h^{-1})	±20.2	±12.9	±17.8	±24.2	±12.4	±18.2	±23.7	±11.7	±20.2

Values are means ± s.e.mean of $n = 13$ healthy female subjects. *$P < 0.05$ *vs* day 1 in ANOVA. Abbreviations: A I, angiotensin I; A II, angiotensin II; A II, angiotensin II; CL_{CR}, creatinine clearance; UVNa, urinary sodium excretion.

Our present finding that administration of 25 mg captopril twice daily for 7 days had no effect on urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ excretion in healthy humans is consistent with the finding of Gerber *et al.* [30] who reported that neither captopril (50 mg twice daily) nor enalapril (10 mg twice daily) for 2 weeks affected urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ excretion in patients with essential hypertension. Although variability of the data was relatively high in our study due to the small sample size, 95% confidence intervals of the changes in prostanoid excretion also did not indicate a trend towards higher values after captopril, suggesting that the failure to detect significant changes was not due to small sample size. However, due to the high variability of our data, minor changes in prostanoid or NO production may have remained undetected.

Low-dose ASA is well known to inhibit platelet cyclo-oxygenase activity and, thus, thromboxane formation relatively selectively [6], although a slight decrease in prostacyclin formation seems to be unavoidable even with very low doses of ASA [22]. This is believed to be due to the inhibitory effect of ASA on the transfer of cyclic endoperoxides from platelets to the endothelium [34]. Therefore, an interaction between acetylsalicylic acid and captopril might be possible, even though low-dose ASA does not directly impair endothelial cyclo-oxygenase activity. However, in our present study we found no interaction of low-dose ASA with the hypotensive effect of captopril. Moreover, although ASA reduced 2,3-dinor-TXB_2 excretion by $>80\%$, it only slightly affected 2,3-dinor-6-keto-$PGF_{1\alpha}$ excretion, and did not affect urinary PGE_2 excretion, an index of renal cyclo-oxygenase activity [35], at all. The absence of any effect of low-dose ASA on urinary PGE_2 in our present study therefore indicated that low-dose ASA induced a presystemic inhibition of platelet cyclo-oxygenase, without any systemic inhibitory effects. This finding confirms earlier observations made by us with similar doses of ASA [22, 23]. Low-dose ASA comedication did not affect the haemodynamic effects of captopril in healthy subjects in our present study. Similarly, van Wijngaarden *et al.* [37] showed that ASA in a single dose of 236 mg did not change the haemodynamic alterations induced by captopril. In contrast, Hall *et al.* [36] demonstrated that a dose of 325 mg ASA, when given on the day before the ACE inhibitor enalapril (10 mg), significantly attenuated the haemodynamic effects of ACE inhibition in patients with chronic stable, but severe heart failure.

A contribution of NO to the vasodilator effects of ACE inhibitors has also been suggested based upon *in vitro* data: Linz *et al.* [38] reported that ramiprilat stimulated cGMP formation in cultured endothelial cells via bradykinin. The NO synthase inhibitor N^{ω}-nitro-L-arginine significantly reduced bradykinin-induced vasodilatation in rabbits [15, 27]. Cachofeiro *et al.* [16] administered captopril either alone or after pre-infusion of N^{ω}-monomethyl-L-arginine in spontaneously hypertensive rats, and found that the NO synthase inhibitor attenuated the hypotensive effect of captopril in this model. In humans evidence suggesting a contribution of NO to ACE inhibitor-induced hypotension has been presented by Hirooka *et al.* [39] who demonstrated that the impaired local forearm vasodilatation to acetylcholine is improved by captopril in hypertensive patients, but not in young healthy volunteers. Furthermore, Hoffmann & Düsing [40] showed that an acute dose of 10 mg lisinopril did not alter intraplatelet levels of cyclic AMP or cyclic GMP, the second messengers of prostacyclin and NO, respectively, in healthy humans. Our present results indicate that captopril neither stimulates basal NO formation (as assessed by urinary NO_3^- excretion rates), nor does it increase its biological activity (as assessed by urinary cyclic GMP excretion), in healthy human subjects.

In conclusion, despite evidence from *in vitro* experimental models suggesting that ACE inhibitors may stimulate endothelial vasodilator autacoid formation by blocking the degradation of bradykinin, medium term administration of captopril in healthy humans did not measurably enhance basal prostacyclin and/or nitric oxide formation *in vivo* in our present study. Low-dose ASA, although exerting a significant inhibitory effect on TXA_2 formation, did not modulate the hypotensive or humoral effects of captopril in healthy human subjects.

The authors gratefully acknowledge the excellent technical assistance of A. Otten. The drugs used in this study were kindly put at our disposition by Schwarz Pharma AG, Monheim, Germany.

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(*Received 7 March 1996, accepted 9 July 1996*)