Aspirin Enhances the Antihypertensive Effect of Captopril in Spontaneously Hypertensive Rats

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SUMMARY  Activation of renal or vascular prostaglandin mechanisms (or both) has been proposed to contribute to the antihypertensive action of captopril. In conscious spontaneously hypertensive rats (SHR) studied in the established phase of hypertension, the blood pressure-lowering effect of captopril, 30 mg/kg/12 hr p.o. given for 7 days, was greatly enhanced by the addition of aspirin, 200 mg/kg/day s.c. Systolic blood pressure decreased from 185 ± 6 and 182 ± 4 to 135 ± 4 and 153 ± 3 mm Hg in rats treated, respectively, with captopril and aspirin or captopril alone, and was unaltered by either vehicle or aspirin alone. Water intake was inconsistently affected by captopril but was increased (p<0.01) by aspirin and was even higher after captopril-aspirin treatment (p<0.01). Urine volume was elevated in all 3 drug-treated groups, increasing threefold after captopril-aspirin treatment. Excretion of sodium and potassium was unchanged by any treatment regimen. In the vehicle group, prostaglandin F₂α, excretion, measured by radioimmunoassay, ranged between 65 and 93 ng/8 hr and was twofold to fourfold higher than that of prostaglandin E₂. Prostaglandin F₂α was unaffected during captopril treatment, whereas prostaglandin E₂ excretion decreased to 12 ± 2 ng/8 hr (p<0.01) by Day 7. Long-term aspirin treatment, either with or without captopril, did not cause sustained inhibition of renal prostaglandin excretion, although a transient effect occurred within the first four hours of administration. These results indicate 1) aspirin potentiates the blood pressure-lowering effect of captopril in SHR, an effect that is associated with a threefold increase in urine flow; 2) this hypotensive action of captopril is independent of a renal prostaglandin mechanism; and 3) the intended effect of cyclooxygenase inhibitors such as aspirin should be validated by measuring prostaglandins in biological fluids. (Hypertension 10: 294-302, 1987)

KEY WORDS  • captopril • prostaglandins • blood pressure • urine volume • plasma renin activity

THE mechanism of the antihypertensive action of captopril, an orally active angiotensin converting enzyme inhibitor,1 cannot be explained entirely in terms of diminished activity of the renin-angiotensin system. The evidence for this statement is well documented. In brief, captopril is effective in experimental models of hypertension and in essential hypertension where the participation of the renin-angiotensin system is not considered to be primary.2-3 Under these circumstances, pretreatment plasma renin activity (PRA) usually does not correlate with the extent of blood pressure fall after captopril treatment, although there is a close relationship between the antihypertensive response and the captopril-induced increments in PRA.4 In the face of angiotensin II receptor blockade,5 subsequent captopril treatment may produce a further decline in blood pressure over that achieved with the antagonist alone and has been shown to be more effective in hypertensive patients than [Sar¹,Thr⁴]angiotensin II, an antagonist with minimal agonist activity.6 Also, there is a lack of a temporal relationship between the degree of converting enzyme inhibition and the hypotensive response.8 Indeed, increased serum and tissue converting enzyme activity with long-term treatment has even been reported.9 Finally, the dose of captopril required to achieve a maximal antihypertensive effect is considerably in excess of that required for converting enzyme inhibition.10 These considerations have prompted a search for additional components of the response to captopril not attributable to a reduction in angiotensin II generation. Not unexpectedly, a role for the kallikrein-kinin system has been envisaged, as converting enzyme is identical to kininase II.11 As both the kallikrein-kinin and
renin-angiotensin systems interact with the prostaglandin system in several ways, prostaglandin-dependent mechanisms may also be involved.

Although a number of animal studies, using a variety of techniques, have addressed the hypothesis that captopril may cause changes in prostaglandin release, the data are conflicting. Furthermore, temporal changes in prostaglandin release during long-term captopril administration have been examined in only one study. We therefore examined the effect of long-term captopril administration with or without aspirin on blood pressure, PRA, urine volume, urine electrolytes, water intake, and hematocrit using conscious male spontaneously hypertensive rats (SHR), a non-renin-dependent model of hypertension, and attempted to relate our findings to alterations in the urinary prostaglandin excretion rate, an index of captopril-induced effects on renal prostaglandin-related mechanisms. To address the effects of converting enzyme inhibition on extrarenal prostaglandin production, we administered aspirin together with captopril to identify prostaglandin-independent effects of captopril in SHR. We found, quite unexpectedly, that the blood-pressure-lowering action of captopril was greatly potentiated by aspirin and was associated with a marked diuresis.

Materials and Methods

Male SHR of the Wistar-Kyoto strain (Charles River Breeding Laboratories, Wilmington, MA, USA), 20 to 40 weeks of age, were used in these experiments in accordance with institutional guidelines. Animals were housed individually in metabolic cages and allowed free access to Purina standard rat chow (St. Louis, MO, USA) except during urine collection. Tap water was given ad libitum.

Protocol 1

Rats were randomly assigned to four drug treatment regimens: captopril, aspirin, captopril and aspirin, and vehicle control. The dose of captopril selected (30 mg/kg b.i.d.) was based on the study by Muirhead et al., who showed that in male SHR rats this dosing regimen resulted in reductions in blood pressure that were evident even 9 hours after dosing. There was no further decline in blood pressure when a dose of 100 mg/kg was administered.

Aspirin was used as it has fewer nonspecific effects than indomethacin, and its use, even at high doses, is associated with a low incidence of gastrointestinal lesions in the rat and does not cause any signs of damage to the kidneys of the rat. Moreover, aspirin has a long duration of action, an obvious practical advantage, as it irreversibly acetylates the cyclooxygenase enzyme complex so that de novo enzyme synthesis is required for recovery from inhibition. When determined ex vivo, prostacyclin synthesis in rat gastric mucosa was inhibited by 85% 24 hours after a single dose (200 mg/kg s.c.) of aspirin and was still inhibited by 61% 72 hours after treatment. Thus, we elected to administer the same dose, which, although high, was deemed necessary to ensure maximal suppression of prostaglandin synthesis, especially as renal cyclooxygenase is relatively resistant to inhibition by nonsteroidal anti-inflammatory drugs (NSAIDs), particularly in the rat, which is 10 times less sensitive to aspirin than, for example, the rabbit.

Captopril (30 mg/kg), dissolved in distilled water (30 mg/ml), was administered by gavage twice daily. Aspirin and vehicle groups received an equivalent volume of distilled water. Aspirin (200 mg/kg), freshly prepared as the sodium salt using an equivalent amount of warmed sodium carbonate solution (100 mg/ml), was injected subcutaneously. A new injection site on either flank was chosen each day. Captopril-treated and vehicle-treated rats received an equivalent amount of sodium solution, at a similar pH, using the sodium bicarbonate salt. All drugs were administered between 0900 and 1000 and the second dose of captopril was given at 2200.

After water intake was recorded, rat blood pressures were measured at random beginning at 1100, after which the animals were weighed and returned to clean cages. Blood pressure readings were taken using a Naturne KN209 blood pressure recording system (Peninsula Laboratories, Belmont, CA, USA). Rats were placed in a thermostatically controlled heating box (36-38°C) for approximately 20 minutes. They were then transferred to a restraining cage kept at room temperature and a cuff, placed on the upper portion of the tail, was inflated to at least 200 mm Hg. The pressure was slowly released, and the return of the systolic pulse, detected by a photoelectric cell, was automatically recorded. The mean of at least three recordings was tabulated. Urine collection began at 1400 and ended 8 hours later at which time the voided volume was measured and the urine samples were transferred to glass vials and stored at −20°C until assayed for prostaglandins. This collection period included 4 hours each of the light and dark cycles. A 4- to 7-day period was allowed to establish reproducible baseline blood pressure, daily water intake, and 8-hour urine volume. Data were obtained for an additional 4 days before the 7-day drug treatment regimen began.

Protocol 2

Unexpectedly, inhibition of urinary prostaglandin excretion, measured 4 to 12 hours after aspirin administration, was not sustained during long-term treatment (see Results). However, this did not rule out the possibility of inhibition within the first 4 hours of drug administration, the time when blood pressure was measured. Therefore, in a subsequent study, we measured changes in urinary prostaglandin F2α (PGF2α) concentrations in samples collected for the first 4 hours immediately after dosing. PGF2α was selected because the data for this prostaglandin were the most anomalous with respect to the effect of aspirin.

Four groups of 7 male SHR were treated with either captopril, captopril and aspirin, aspirin, or vehicle as in Protocol 1 for 4.5 days. No acclimatization period was included. Urines were collected for 24 hours, di-
vided in three periods, from 1000 to 1400, 1400 to 2200, and 2200 to 1000. At 1400 blood for hematocrit determinations was collected from the tip of the tail.

On the fifth day, just before being killed, rats were immobilized in a soft cloth and 1 ml of tail blood was collected into microfuge tubes containing 40 µl of EDTA (70 mg/ml) for determinations of PRA. After centrifugation, the plasma was stored at −20°C until assayed.

**Prostaglandin Radioimmunoassay**

Measurement of urinary concentrations of immunoreactive PGF$_2\alpha$ (irPGF$_2\alpha$) and prostaglandin E$_2$ (irPGE$_2$) was performed according to Dray et al. without sample purification. Details of the assay and antibody cross-reactivities have been described elsewhere. Non-specific binding of randomly selected urines from the four treatment groups was less than 3%. The use of unextracted urines was validated in several ways, as recommended by Granstrom and Kindahl and Midgley et al. First, antibody binding curves for serially diluted urines paralleled those of PGF$_2\alpha$ and PGE$_2$ standards. Second, addition of known quantities of irPGF$_2\alpha$ and irPGE$_2$ measured in acid-lipid-extracted or Sep-Pak (Waters Associates, Milford, MA, USA) purified urines (using three different antisera for PGF$_2\alpha$) were the same as those in crude samples. Radioimmunoassay (RIA) of prostaglandins using unextracted rabbit urine has been validated previously.

**PRA–Angiotensin I Radioimmunoassay**

Angiotensin I (ANG I) was measured using the Squibb ANG I RIA kit (Princeton, NJ, USA). Plasma samples were incubated in duplicate for 1 hour at 37°C to reflect the amount of ANG I generated through the action of renin, at 4°C to reflect the circulating levels of ANG I. Generation in the 37°C samples was stopped by transferring the tubes to the ice bath. Samples were stored at −20°C until assayed. After the appropriate dilutions using 0.1 M phosphate buffer, pH 6, samples were assayed according to kit instructions. Radioactivity was measured in a Beckman gamma counter (Model 4000; Somerset, NJ, USA). Preliminary studies showed that ANG I generation, in all four treatment groups, was linear for at least 2 hours (data not shown).

**Electrolytes**

Urinary sodium and potassium were measured by autoanalytical flame ionization photometry (Model 1; Technicon, Tarrytown, NY, USA) calibrated against an internal lithium standard.

**Statistical Analyses**

Statistical significance of the differences were evaluated by multifactor analysis of variance for repeated measures using the BMDP2V computer program. PRA data were tested by one-way analysis of variance. Where the F value indicated overall significance, specific comparisons of the means were made using the critical difference computed using the treatment mean square within-group error term obtained in analysis of variance and Student's t value appropriate for the degrees of freedom of the error term.

**Materials**

All reagents and solvents were of analytic grade. Prostaglandin standards were kindly supplied by Dr. J. Pike (Upjohn Company, Kalamazoo, MI, USA); captopril by Dr. Z. P. Horovitz (Squibb Institute, Princeton, NJ, USA); PGF$_2\alpha$ antisera from Dr. J. Quilley of this department. PGE$_2$ antiserum and aspirin were purchased from Sigma (St. Louis, MO, USA).

**Results**

**Protocol 1**

Body weights showed no significant differences among any of the four groups in the pretreatment period or during treatment with vehicle, captopril, or aspirin and ranged between 349 ± 5 and 361 ± 8 g. However, body weight was reduced in the captopril-aspirin group, decreasing to 338 ± 6 g by Day 7, and was significantly lower (p<0.01) than the vehicle group by Day 3.

**Blood Pressure, Water Intake, Urine Volume, and Electrolyte Excretion**

Captopril-aspirin treatment of SHR had the greatest antihypertensive effect, causing a precipitous decline in blood pressure that was significantly greater (p<0.01) than that of the other groups on all days (except that of the captopril group on Day 6); blood pressure had fallen to 136 ± 6 mm Hg by Day 4 (Figure 1). In pilot studies, where blood pressure was measured directly from an indwelling cannula placed in the lower abdominal aorta, the same potentiation of the blood pressure-lowering effect of captopril by aspirin was observed (data not shown). Captopril caused the expected reduction in blood pressure, which was lower (p<0.01) than that in the vehicle-treated and aspirin-treated groups from Day 2 onwards and reached a nadir of 155 ± 6 mm Hg by Day 4 (see Figure 1). Neither vehicle nor aspirin treatment affected blood pressure, which ranged from 183 ± 6 to 187 ± 4 and 184 ± 4 to 188 ± 7 mm Hg, respectively. Mean pretreatment blood pressures ranged between 176 ± 4 and 190 ± 4 mm Hg and were not significantly different among the four groups for any given day.

Daily water intake was unchanged by vehicle treatment and was increased (p<0.05) only on Days 4 and 6, in the captopril-treated group (Figure 2). Water consumption was elevated in both aspirin-treated groups. Intake was increased (p<0.01) to 53 ± 4 ml/day on Day 2 in the captopril-aspirin-treated group and to 51 ± 3 ml/day by Day 2 in the aspirin-treated rats.
ASPIRIN ENHANCES THE EFFECT OF CAPTOPRIL/Quilley et al.

Thereafter, the dipsogenic effect of aspirin alone persisted, while a significantly \((p < 0.01)\) greater increase from the aspirin-treated group, to a maximum of \(71 \pm 3 \text{ ml/day on Day 4,} \) was observed in the captopril-aspirin-treated group (see Figure 2).

The volume of urine excreted in 8 hours was not affected by the vehicle and ranged between 2.7 and 3.2 ml/8 hr (Figure 3). Captopril caused an increase \((p < 0.01)\) in volume from the first day, to 5.3 \(\pm 0.7 \text{ ml/8 hr, which was maintained throughout the 7-day study period. Urine volume also increased, though not always with statistical significance, in the aspirin-treated group, and ranged from 3.9 \(\pm 0.6 \) to 4.8 \(\pm 0.4 \text{ ml/8 hr. As with blood pressure and prostaglandin excretion, the greatest change in urine volume was seen in the captopril-aspirin-treated group, in which a stepwise increase was observed reaching 9.2 \(\pm 0 \text{ ml/8 hr by Day 5, that was higher \((p < 0.01)\) than levels in the captopril-treated group from Day 2 onward (see Figure 3).} \)

No statistically significant alterations in either sodium or potassium excretion were evident with any of the four treatment regimens. Potassium excretion, which varied from 0.43 \(\pm 0.04 \) to 0.69 \(\pm 0.06 \text{ mEq/8 hr, was generally twice that of sodium excretion, which was between 0.21 \(\pm 0.04 \) and 0.41 \(\pm 0.07 \text{ mEq/8 hr.} \)

Urinary Prostaglandin Concentration and Excretion Rates

Prostaglandin concentrations (Table 1) and excretion rates (Figure 4) in the four groups were not different during the pretreatment period and were not affected by the vehicle. \(\text{irPGF}_{2\alpha} \) excretion, which ranged between 65 \(\pm 5 \) and 94 \(\pm 13 \text{ ng/8 hr in the vehicle-treated group, was the major urinary prostaglandin excreted, being twofold to fourfold greater than that of \(\text{irPGE}_2 \), which ranged between 18 \(\pm 3 \) and 28 \(\pm 7 \text{ ng/8 hr.} \)

Despite reductions in \(\text{irPGF}_{2\alpha} \) concentration in captopril-treated rats (see Table 1), increased urine volume resulted in \(\text{irPGF}_{2\alpha} \) excretion rates (see Figure 4) that equaled those in vehicle-treated rats. Urinary \(\text{irPGE}_2 \) concentration and excretion rate were unaffected by captopril treatment on Days 1 and 3. Thereafter, significant \((p < 0.01)\) stepwise decreases occurred, and \(\text{irPGE}_2 \) excretion rate had fallen to 12 \(\pm 2 \text{ ng/8 hr by Day 7 (see Figure 4).} \)

Unexpectedly, long-term aspirin treatment in either group failed to cause a sustained decrease in, and even increased, prostaglandin excretion when the effect was measured 4 to 12 hours after dosing. Thus, the reduced \(\text{irPGF}_{2\alpha} \) urine concentration on Day 1 was offset by the increase in urine volume, and on subsequent days, when \(\text{irPGF}_{2\alpha} \) concentration had returned to control levels, \(\text{irPGF}_{2\alpha} \) excretion rate increased further, reaching 154 \(\pm 13 \text{ ng/8 hr on the fifth treatment day.} \) \(\text{irPGE}_2 \)
TABLE 1. Immunoreactive Prostaglandin Concentration in Urine Collected 4 to 12 Hours After Treatment of SHR with Vehicle, Captopril, Captopril and Aspirin, or Aspirin

<table>
<thead>
<tr>
<th>Treatment day</th>
<th>Vehicle</th>
<th>Captopril</th>
<th>Captopril + aspirin</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>irPGF$_2$ (ng/ml)</td>
<td>$27.4 \pm 2.7$</td>
<td>$26.4 \pm 2.9$</td>
<td>$26.1 \pm 2.5$</td>
<td>$24.4 \pm 4.6$</td>
</tr>
<tr>
<td>irPGE$_2$ (ng/ml)</td>
<td>$7.3 \pm 1.6$</td>
<td>$8.1 \pm 1.2$</td>
<td>$7.8 \pm 1.8$</td>
<td>$7.0 \pm 1.8$</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 8–10). ir = immunoreactive; PG = prostaglandin.

* $p<0.01$, compared with vehicle-treated group; $\dagger p<0.05$ or better compared with captopril-treated group; $\ddagger p<0.05$, compared with aspirin-treated group.

concentration and excretion rate in the aspirin-treated rats were reduced ($p<0.05$) on Day 1 only.

Likewise, aspirin treatment did not inhibit irPGF$_2$ excretion when combined with captopril despite reduced irPGF$_2$ urine concentrations (see Table 1 and Figure 4). Instead, an increase in excretion rate was seen on Days 1, 5, and 7 relative to that of both the vehicle-treated and captopril-treated groups. Capto-

pril-aspirin administration caused a diminution in irPGE$_2$ urine concentration ($p<0.01$) when compared with that of the vehicle-treated group on all days (see Table 1). However, the increase in urine volume in captopril-aspirin-treated rats countered this effect, so that the irPGE$_2$ excretion rate did not differ from that of the vehicle-treated rats on Days 1 and 5. By the seventh treatment day irPGE$_2$ excretion was greater ($p<0.01$) than that of the captopril-treated group (see Figure 4).

Based on these results, changes in prostaglandin excretion (measured between 1400 and 2200 hours; i.e., beginning 4 hours after aspirin treatment) within the various treatment groups can be reduced to several general observations. Captopril caused a late reduction in irPGE$_2$ excretion, being evident by the fifth day. Aspirin did not cause a sustained reduction in prostaglandin excretion. Urinary prostaglandin concentrations were reduced on Day 1 only. On subsequent days, the large increase in urine volume resulted in enhanced prostaglandin excretion in the face of continued administration of aspirin. Finally, the addition of captopril to aspirin treatment also did not result in sustained reduction in prostaglandin excretion, as the reduced urinary concentrations of irPGE$_2$ and irPGF$_2$ were offset by diuresis.

Protocol 2

Prostaglandin Excretion

In the second study, prompted by the lack of sustained inhibition of renal prostaglandin synthesis as reflected in increased prostaglandin excretion from 4 to 12 hours after aspirin treatment, we measured irPGF$_2$ excretion from 0 to 4 hours after administration of aspirin or captopril (or both). The excretion of irPGF$_2$ in 4 hours by vehicle-treated and captopril-treated rats was half that excreted in 8 hours (Protocol 1; Table 2; see Figure 2). When measured in the first 4 hours immediately after aspirin administration,
Excretion Rate of Immunoreactive Prostaglandin $F_{2a}$ and Urine Volume Within the First 4 Hours of Drug Treatment and Daily Urine Volume, Water Intake, and PRA of SHR Studied in Protocol 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Vehicle</th>
<th>Captopril</th>
<th>Captopril + aspirin</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>irPGF$_{2a}$ (ng/4 hr)</td>
<td>Day 1</td>
<td>13.3 ± 2.5</td>
<td>18.6 ± 3.4</td>
<td>5.3 ± 1.9*†</td>
<td>5.3 ± 1.2*†</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>39.7 ± 9.1</td>
<td>37.4 ± 8.1</td>
<td>8.6 ± 2.6*†</td>
<td>8.8 ± 3.5*†</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>32.5 ± 6.6</td>
<td>29.2 ± 7.2</td>
<td>6.9 ± 2.8*†</td>
<td>12.6 ± 2.9*†</td>
</tr>
<tr>
<td></td>
<td>Day 4</td>
<td>37.3 ± 2.7</td>
<td>29.8 ± 5.0</td>
<td>16.5 ± 4.2</td>
<td>33.1 ± 10.4</td>
</tr>
<tr>
<td>Urine volume (0-4 hr (ml/4 hr))</td>
<td>Day 1</td>
<td>1.0 ± 0.3</td>
<td>1.4 ± 0.3</td>
<td>2.9 ± 0.5§</td>
<td>3.2 ± 0.6*§</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>1.6 ± 0.2</td>
<td>1.8 ± 0.3</td>
<td>3.6 ± 0.7*§</td>
<td>2.7 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>1.4 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>3.8 ± 1.2*†</td>
<td>3.4 ± 0.8*†</td>
</tr>
<tr>
<td></td>
<td>Day 4</td>
<td>1.9 ± 0.3</td>
<td>1.4 ± 0.1</td>
<td>4.4 ± 1.2*†</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>Urine volume (0-24 hr (ml/24 hr))</td>
<td>Day 1</td>
<td>6.7 ± 1.3</td>
<td>9.5 ± 1.4</td>
<td>19.7 ± 2.5*†</td>
<td>11.4 ± 1.3*</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>7.8 ± 1.2</td>
<td>9.0 ± 1.2</td>
<td>23.8 ± 3.1*†</td>
<td>10.0 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>7.5 ± 1.1</td>
<td>8.4 ± 1.3</td>
<td>24.0 ± 2.7*†</td>
<td>12.0 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Day 4</td>
<td>8.5 ± 1.3</td>
<td>10.5 ± 1.2</td>
<td>25.0 ± 3.0*†</td>
<td>14.9 ± 2.4*</td>
</tr>
<tr>
<td>Water intake (ml/24 hr)</td>
<td>Day 1</td>
<td>35 ± 6.3</td>
<td>40 ± 3.3</td>
<td>47 ± 4.1*∥</td>
<td>36 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>38 ± 3.2</td>
<td>35 ± 2.7</td>
<td>50 ± 5.2*∥</td>
<td>42 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>35 ± 3.6</td>
<td>38 ± 2.0</td>
<td>58 ± 4.5*∥</td>
<td>45 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>Day 4</td>
<td>37 ± 1.7</td>
<td>40 ± 0.9</td>
<td>58 ± 4.4*∥</td>
<td>49 ± 2.7</td>
</tr>
<tr>
<td>PRA (ng ANG I/ml/hr)</td>
<td>Day 5</td>
<td>11.8 ± 2.0</td>
<td>48.8 ± 8.2*</td>
<td>44.4 ± 4.9∥</td>
<td>14.0 ± 2.9</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 6–7). irPGF$_{2a}$ = immunoreactive prostaglandin F$_{2a}$.

* p < 0.01, † p < 0.05, compared with vehicle-treated group.

Table 2.

Discussion

The primary objective of this study, to identify a possible dependency of the antihypertensive action of captopril on a renal prostaglandin mechanism, was answered in the negative. Captopril lowered blood pressure in the SHR independently of augmented renal prostaglandin release, as reflected by urinary prostaglandin excretion. In fact, a late effect of captopril, evident by the fifth day, was to diminish prostaglandin excretion in the face of a sustained antihypertensive effect. We also combined aspirin with captopril treatment in an attempt to nullify any prostaglandin-related action of captopril, such as an extrarenal mechanism, that might not have been revealed by changes in prostaglandin excretion. The finding that aspirin enhanced the blood pressure-lowering effect of captopril was unexpected, although it was explained by the notable diuresis induced by the combined administration of captopril and aspirin. In addition, the renal action of aspirin on prostaglandin synthesis, either alone or in combination with captopril, was transient and was succeeded by increased excretion of prostaglandins despite continued administration of high dose aspirin. A sustained systemic effect of aspirin could be discerned,
however, as potentiation of the antihypertensive action of captopril was maintained, which, as noted, was related to the diuretic effect of the drug combination. The sustained potentiation of the blood pressure-lowering effects of captopril by aspirin and the associated diuresis are crucial, as they indicate that neither the route of administration of aspirin nor its rapid elimination in the rat prevented a long-term effect of aspirin, despite the transient effects on renal prostaglandin excretion. Since this study was completed, we have shown that, unlike aspirin, other NSAIDs can produce sustained inhibition of renal prostaglandin release in the rat. Paradoxically, when tissues of the SHR were studied ex vivo, a sustained inhibition of cyclooxygenase activity in response to aspirin was found. Again, this effect was not reflected in changes in urinary prostaglandin excretion. The failure of aspirinlike drugs to inhibit renal prostaglandin release in the conscious animal is not unprecedented, as renal venous efflux of prostaglandins in conscious dogs was unaffected by indomethacin, and long-term indomethacin failed to inhibit urinary prostaglandin excretion in rats with diabetes insipidus infused with 1-desamino-8-D-arginine vasopressin.

The finding that captopril exerts an antihypertensive action independently of enhanced renal prostaglandin release is in agreement with the report of unchanged urinary prostaglandin excretion during long-term captopril treatment of SHR. Further, studies in normotensive animals did not disclose an effect of converting enzyme inhibition on either urinary or venous PGE_2 levels. On the other hand, elevated excretion of PGE_2 usually has been reported as an acute response in human studies. The antihypertensive response to captopril was either unaffected or reduced when the effect of concomitant NSAID administration was examined. More to the point, in SHR the antihypertensive action of captopril was not modified by either aspirin, administered at the same dose as we used (200 mg/kg/4 hr s.c.) or by indomethacin. However, aspirin-induced potentiation of the blood pressure-lowering effect of captopril in SHR has been described by Mullane et al., who used the same doses of each drug as in our study. In addition, the potentiated antihypertensive response was accompanied by increased urine volume. In another study, blood pressure of SHR treated with captopril and indomethacin was reduced by 43 mm Hg versus a drop of 24 mm Hg in rats treated with captopril alone. Further, the development of hypertension in young SHR was prevented by aspirin administration. As noted, enhancement of the response to captopril by aspirin is unlikely to be due to alterations in renal prostaglandin release. We obtained additional evidence that aspirin given in high doses did not inhibit renal prostaglandin synthesis under our experimental conditions in the SHR. Thus, the elevated PRA produced by captopril was not affected by aspirin, whereas indomethacin has been reported to lower captopril-induced elevations in PRA.

In captopril-aspirin–treated rats, the potentiating effect of aspirin on the antihypertensive effect of captopril appears to result primarily from a reduction in extracellular fluid volume. Thus, the temporal relationship between increased urine volume observed on the first day and the increase in water intake evident from the second day strongly suggests that increased drinking was secondary to changes in water excretion. Moreover, the weight loss, which was not associated with any gross alteration in food intake or in fecal composition, and the slight, though not significant, increase in hematocrit provide additional evidence for overall water loss. Because potassium excretion was unchanged, the polyuria cannot be attributed to hypokalemia resulting from toxicity of aspirin. Indeed, long-term administration of this dose of aspirin did not cause renal damage in the rat.

As urine volume increased without changes in electrolyte excretion, an inhibitory effect of aspirin on antidiuretic hormone (ADH) may account for the augmented antihypertensive response. A prostaglandin-dependent mechanism has been proposed to participate in regulating ADH release, as intracerebroventricular administration of PGE_2 has been shown to increase ADH release and indomethacin inhibited osmotically stimulated ADH release. Thus, the greater reduction in blood pressure and increase in urine volume in the captopril-aspirin–treated group may be related to diminished prostaglandin-dependent ADH release. The failure of aspirin to reduce renal prostaglandin excretion, except acutely, does not exclude this possibility. Long-term aspirin treatment may still be acting centrally to inhibit ADH release in view of differences in the susceptibility of various tissues of the same species to cyclooxygenase inhibitors. Moreover, in a recent study we found a continued suppression of ex vivo prostaglandin synthesis in aorta, spleen, and renal papilla in response to long-term aspirin administration.

Centrally acting prostaglandin-dependent mechanisms elevate blood pressure, an effect that is greater in SHR than in Wistar-Kyoto rats. Thus aspirin-induced inhibition of these central mechanisms could be expected to lower blood pressure. Further, captopril may influence the activity of peptides that participate in central mechanisms of blood pressure regulation and that interact with prostaglandins, such as kinins. Thus, sustained inhibition of prostaglandin synthesis within the brain could contribute to the enhanced antihypertensive response to captopril when aspirin is coadministered.

It is equally possible that the greater reduction in blood pressure of SHR treated with captopril-aspirin may be unrelated to changes in prostaglandin synthesis. Instead, it may arise from a pharmacokinetic interaction. The reasons for this are twofold: the organic acid secretory pathway of the kidneys is a major route for the elimination of both captopril and aspirin, and both drugs are extensively bound to plasma proteins. Thus, prevention of captopril elimination by competition for renal secretion and displacement from protein binding sites may result in increased free captopril. There are two reasons for discounting this possibility. First, the dose of captopril chosen probably produced a
maximal fall in blood pressure, as the response was equal to that reported by Muirhead et al., who showed no further blood pressure reduction in SHR treated with larger doses. Second, in the presence of probenecid, an inhibitor of organic acid secretion, plasma levels of captopril were not altered because elimination through hepatic metabolism was increased.

In summary, aspirin greatly potentiated the antihypertensive action of captopril in SHR, an effect associated with diuresis. Our findings argue against the participation of renal prostaglandins in the antihypertensive action of captopril. The unexpected failure of long-term high dose aspirin treatment to cause sustained inhibition of renal prostaglandin release in vivo demonstrates the need to verify the intended effect of cyclooxygenase inhibitors by measuring prostaglandin levels in biological fluids.

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