The Antihypertensive Function of the Kidney
Its Elucidation by Captopril plus Unclipping

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SUMMARY Unclipping the one-kidney, one-clip hypertensive rat during a free flow of urine caused the blood pressure to return to normal levels within about 3 hours. We found that administration of captopril plus unclipping caused the blood pressure to return to normal in minutes (17 ± 4). Ureterocaval anastomosis plus captopril plus unclipping also caused the blood pressure to return to normal in minutes (8.8 ± 2). Thus, the potentiation of the drop in blood pressure does not seem to be due to a volume effect. Administration of indomethacin and aprotinin did not prevent a rapid decline of the blood pressure after unclipping, but the decline was less rapid than that occurring after captopril and unclipping, which suggests that prostaglandin may have some effect on this mechanism. Saralasin administration did not potentiate the antihypertensive action of captopril plus unclipping. Chemical papillectomy prevented the drop in blood pressure after unclipping. The bolus dose of captopril to the hypertensive rat often caused a transient depressor effect resembling that due to the antihypertensive neutral renomedullary lipid, which suggests secretion of this lipid into the blood. The renomedullary interstitial cells accumulated large lipid granules after captopril administration. These cells also degranulated after unclipping. These findings are consistent with the hypothesis that the renal papilla secretes an antihypertensive hormone after unclipping. At present, antihypertensive neutral renomedullary lipid is the main putative hormone.

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KEY WORDS unclipping • captopril • renal papilla • indomethacin • aprotinin • saralasin • antihypertensive neutral renomedullary lipid • one-kidney, one-clip hypertension • antihypertensive hormone

REMOVAL of the clip constricting the renal artery of the two-kidney, one-clip hypertensive rat lowers the blood pressure (BP) to normal within 12 to 24 hours.1 According to recent data, this effect results from decreasing the peripheral vascular resistance.1,3 This decrease may result from the renal papilla secreting depressor substance(s)6-7 that is neither prostaglandin nor kinin.8 Moreover, blockade of the renin-angiotensin system does not alter the recession of the BP after unclipping.2 Thus, three separate systems (prostaglandin, kinin, and renin-angiotensin), according to these reports, do not appear to be involved in the antihypertensive function of the kidney following unclipping of the two-kidney, one-clip hypertensive rat.

Unclipping the one-kidney, one-clip hypertensive rat has caused some controversy, although it has been shown to lower the BP to normal within 24 hours.9 Ledingham and Cohen10 reported that the BP recession was attended by decreased cardiac output followed by decreased peripheral vascular resistance (the opposite of the Ledingham-Guyton prohypertensive sequence11,12). Liard and Peters13 noted a diuresis-natriuresis following unclipping of the one-kidney model and ascribed the lowering of the BP to this phenomenon. To support their contention, these workers found the BP did not return to normal following ureteral ligation and unclipping (no loss of sodium and water). Other reports have shown that ureteral ligation and its attendant hydronephrosis interfere with the antihypertensive function of the kidney.14,15 In addition, neither replacement of sodium-volume loss16,17 nor deviation of the urine back into the system through a vein16,18 prevents the lowering of the BP to normal after unclipping the one-kidney, one-clip rat. Moreover, the BP also has been shown to return to normal despite a major fluid load.16

These findings suggest that unclipping lowers the BP through mechanisms seemingly unrelated to known renal activities (i.e., sodium-volume control,
The purpose of this study was to further elucidate the mechanism(s) involved in the lowering of the BP following unclipping the one-kidney, one-clip hypertensive rat, particularly through the use of captopril before unclipping. Our experiments were based on the hypothesis that the BP is lowered after unclipping by an antihypertensive hormone — antihypertensive neutral renomedullary lipid (ANRL) — secreted by the renal papilla.

Methods

Animals

The six-week-old male Sprague-Dawley rats, obtained from one supplier (Bio Lab, St. Paul, MN), weighed 200 to 220 g. The rectangular clip with a gap of 0.279 mm was applied to the left renal artery, and the right kidney was removed, as previously described. After 3 to 6 months, the rats weighed 430 to 566 g (average, 470 g) and the BP was 150 to 210 mm Hg (average, 181 mm Hg).

Unclipping

Our method of unclipping differs from that of others. Pentobarbital is injected intravenously (30 mg/kg), which often causes a transient lowering of the BP (average, -25 mm Hg) that lasts an average of 30 minutes. During this time, when the animal is under maximal effect of the anesthetic, the abdominal incision is made and the clip is exposed and prepared for removal. As soon as the BP returns to or near the original hypertensive level, the clip is removed. The rate of drop of the BP in millimeters of mercury per minute (ΔBP/Δt) was determined by the difference between the control hypertensive BP and the BP at the nadir following unclipping over time in minutes. The nadir was defined as the leveling BP after unclipping.

Drugs

An indwelling catheter in the inferior vena cava was used to infuse all drugs. To produce chemical papillectomy, bromethylamine hydrobromide was injected in 1 ml of saline as a single i.v. bolus (250 mg/kg) 8 to 9 days before the experiment while the clip was in place and the animals were hypertensive. This approach represents a departure from that used by others. The latter workers gave the drug before the clip was applied. It was determined by electron microscopy that the papilla was necrotic after the 8- to 9-day interval.

Captopril (kindly furnished by Dr. Zolla Horovitz of the Squibb Institute for Medical Research, Princeton, NJ), the converting-enzyme inhibitor, was injected (10 mg/kg) as a single i.v. bolus to a majority of the animals (35 to 46). The others received the compound intramuscularly (30 mg/kg in 6; 3–20 mg/kg in 5).

Saralasin (Sigma Chemical Co., St. Louis, MO) was infused intravenously in saline by a Sage pump (Sage Instruments Inc., Cambridge, MA) at the rate of 12 µg/kg/minute for 3½ hours (30 minutes before unclipping to ensure no change in BP occurred). The total volume of saline infused was 1.2 to 1.4 ml.

Two groups of experiments used indomethacin (kindly furnished by Dr. C. A. Stone of Merck, Sharp & Dohme Co., West Point, PA) and aprotinin (Sigma Chemical Co.). In the first group, indomethacin was injected subcutaneously in sesame oil (7.5 mg/kg per dose), as recommended by Colina-Chourio et al. for the rabbit. Aprotinin (50,000 trypsin-inhibiting units) was injected intramuscularly in 0.5 ml of saline per dose. Two doses of indomethacin and aprotinin were given, one about 14 hours before the experiment and one about 90 minutes before the captopril injection. The dose of indomethacin was shown previously to elevate the plasma indomethacin level to approximately 20 µg/ml. This first group received both compounds at different sites. The second group was given indomethacin or aprotinin alone before unclipping.

Electron Microscopy

At the end of the experiments with captopril and chemical papillectomy the renal papilla was removed, prepared, and studied by electron microscopy as previously described. In addition, morphometric studies were conducted on the renomedullary interstitial cells (RIC) with emphasis on volume density of the lipid granules of the RIC, granule volume per volume of RIC, and granules per square millimeter of tissue section.
Urine Studies

Sodium and potassium excretion was measured in an Astra 8 (Beckman Co., Fullerton, CA). Prostaglandins (PG E2 and 6-keto-PGF1α, the metabolite of PGI2) levels were measured by radioimmune assay.

Experimental Groups

Group I, which consisted of administration of captopril plus unclipping, comprised five subgroups, two of which were controls. All the animals were prepared for unclipping as previously described.

Captopril was injected in Subgroup A (n = 12), and 30 to 55 minutes later (this interval was dependent on whether the bolus dose of captopril evoked an acute depressor effect of the ANRL type; see Figure 7), when the BP remained steady near its preexperiment hypertensive level, the clip was removed and urine was allowed to flow freely.

In Subgroup B (n = 12) the ureter was anastomosed to the inferior vena cava, as previously described. Thus, there was no flow of urine to the outside of the body. This procedure required an average of 15 minutes. Captopril then was given, and unclipping proceeded as described for Subgroup A. With each ureterocaval anastomosis experiment (this group and its control, Subgroup D), the catheter in the vena cava was removed at the end of the experiment and a free flow of urine was demonstrated. This maneuver represents an essential control, as blockage of the ureter and its attendant hydronephrosis interfere with the antihypertensive function of the renal papilla.

Subgroup C (n = 10) was the first control group, receiving neither captopril nor saralasin. The clip was removed, and urine was allowed to flow freely as the BP was monitored.

Subgroup D (n = 9) was the second control group (no captopril, no saralasin). Ureterocaval anastomosis was established, and the clip was removed. The BP was monitored for 5 hours. The results in this group have been previously reported. All other groups contain new data.

Subgroup E (n = 12) was the third control group. Captopril was injected, and the BP was monitored for 3 to 5 hours without unclipping. The pressor response to angiotensin I (50 ng i.v.) was determined before and each hour after the captopril administration and was shown to be blocked.

The pressor effect of 50 ng of angiotensin II (Ang II) i.v. was established (average +43 mm Hg), then the saralasin infusion was started in Group II (n = 10). After 30 minutes it was shown that the pressor effect of Ang II was blocked and that the BP had not changed significantly (181 ± 3 mm Hg before and 177 ± 2 mm Hg after the saralasin; p > 0.2). At this time, the clip was removed and the BP was followed for 3 hours while the saralasin infusion was continued. Blockage of the pressor effect of Ang II was noted after each elapsed hour.

Subgroup A of Group III (n = 6) was given indomethacin plus aprotinin. The first dose of indomethacin and aprotinin dropped the BP overnight but not quite to significance (p > 0.05). The second dose caused a transient vasodepressor effect that lasted an average of 89 minutes. Captopril i.v. also caused a transient vasodepressor effect (average 93 minutes). Unclipping occurred after the BP had stabilized at its overnight hypertensive level (165 ± 7 mm Hg).

Subgroup B of Group III received indomethacin (n = 6) and aprotinin (n = 6) separately. The same two doses of indomethacin and aprotinin were given about 14 hours apart to separate animals to determine if the results would differ from those obtained when the compounds were injected at the same time.

Group IV (n = 12), which underwent chemical papillectomy with bromethylamine hydrobromide plus unclipping, was divided into animals receiving captopril (n = 6) and animals not receiving captopril (n = 6) before unclipping. There was no ANRL-like vasodepressor effect after captopril administration.

Statistics

Statistical analysis was conducted by t test and analysis of variance (ANOVA plus LSD).

Results

Group I

There was no difference between the preexperiment BP (taken the week before) and the BP at the time of the experiment for Subgroups A, B, and C of Group I: Subgroup A, 185 ± 5 and 181 ± 6 mm Hg (p > 0.6); Subgroup B, 178 ± 3 and 171 ± 3 mm Hg (p > 0.2); and Subgroup C, 181 ± 3 and 177 ± 2 mm Hg (p > 0.2). After unclipping, the BP in Subgroup A (captopril, unclipping, free flow of urine; Figure 1) reached 120 ± 8 mm Hg in 17 ± 4 minutes (p < 0.001 versus hypertensive BP). In four animals the BP was monitored for 3 hours and remained depressed the entire time (Figure 2A). The BP remained depressed at 24 hours. After unclipping, the BP in Subgroup B (ureterocaval anastomosis, captopril, unclipping; see Figure 1) reached 136 ± 4 mm Hg in 8.8 ± 2 minutes (p < 0.001). The BP in Subgroup C (no captopril, unclipping, free flow of urine; see Figure 1) dropped from 180 ± 3 to 130 ± 2 mm Hg in 160 ± 13 minutes (p < 0.001) after unclipping. After unclipping, the BP in Subgroup D (no captopril, ureterocaval anastomosis, unclipping; see Figure 1) changed from 190 ± 6 to 163 ± 4.5 mm Hg in 5 hours. The BP of this group returned to normal after 45 to 50 hours. In Subgroup E (captopril without unclipping; Figure 3), captopril administration alone caused a slight depression of the BP (184 ± 6 to 168 ± 6 mm Hg) that lasted 1 to 2 hours. Thereafter, the BP was no different from the prior hypertensive level. Thus, the single dose of captopril had no lasting effect on the BP.

Group II

After unclipping, the BP of Group II (saralasin, unclipping, free flow of urine; Figure 4) dropped to 133 ± 6 mm Hg at 90 minutes, 128 ± 4 mm Hg at 2 hours, and 122 ± 4 mm Hg at 3 hours. These changes
The four slopes, indicating the rate of decline of the mean BP after unclipping (UC) of the one-kidney, one-clip rat, resulted from captopril plus UC and the free flow of urine (FFU), captopril plus ureterocaval anastomosis (UCA), and both procedures without captopril. The main comparisons are between C + UCA + UC and UCA + UC and between C + FFU + UC and FFU + UC. Captopril greatly potentiated the antihypertensive function of the kidney after UC. The −4 days indicates that the hypertensive BP was derived as an average over 4 days before the experiment. The −30 minutes indicates that captopril was injected 30 minutes before the experiment. The same format is used in Figures 3, 4, and 7.

Figure 2. Transient depressor effect of captopril in one-kidney, one-clip murine hypertension. A. Effect on a one-kidney, one-clip hypertensive rat subjected to captopril plus unclipping (UC) plus free flow of urine (FFU). The hypertensive mean BP was 185 mm Hg. The first injection of angiotensin II (A; 50 ng i.v.) evoked the expected pressor response (∼+65 mm Hg). This pressor response was blocked at the second, third, and fourth injection because of the captopril (C) given. Unclipping caused a rapid decline of the BP (from 185 to −115 mm Hg in ∼6 minutes). Thereafter, the BP remained about 125 mm Hg for the 3 hours of observation. In other examples the BP remained depressed at 24 hours. B. The transient acute vasodepressor effect following i.v. captopril. There was a lag of approximately 2 minutes followed by a decline in BP (175 to ∼140 mm Hg). Recovery occurred after 14 minutes. Approximately 15 minutes after UC plus FFU, the BP dropped from 175 to approximately 115 mm Hg.
were different from those of Subgroups A and B in Group I but did not quite reach significance when compared with Subgroup C (no captopril, unclipping, free flow of urine; 0.1 < p > 0.05). Thus, the results with saralasin differed from those with captopril.

Group III

The first dose of indomethacin and aprotinin in Group III, Subgroup A (indomethacin and aprotinin, unclipping, free flow of urine; Figure 5) lowered the BP from 182 ± 5 to 165 ± 6 mm Hg (p > 0.05, not significant at this level). The second dose caused a transient vasodepressor effect lasting an average of 89 minutes. Captopril also caused a transient depressor effect lasting 1 hour in five rats and 2½ hours in one rat (Figure 6). This is the second group displaying this phenomenon following i.v. captopril. Following unclipping (see Figure 5), the BP dropped to 138 ± 9 mm Hg in 15 minutes (p < 0.05), to 128 ± 12 mm Hg in 30 minutes (p < 0.02), and to 122 ± 3 mm Hg in 45 minutes (p < 0.01). Blockage of prostaglandin synthesis was indicated by no immunoreactive PGE2 in the urine before and after unclipping and a marked depression of urinary 6-keto-PGF1α.

In Subgroup B of Group III (indomethacin and aprotinin given alone before captopril, unclipping, and free flow of urine) neither indomethacin nor aprotinin given as a single agent had an effect on the hypertensive BP: 199 ± 3 and 203 ± 4 mm Hg before and 198 ± 5 and 198 ± 6 mm Hg after administration respectively (each n = 6; p > 0.5–0.7). The rate of BP decline for each group receiving the compounds separately plus captopril and unclipping was similar to that when the compounds were given together (together ΔBP/Δt = 1.6 ± 0.4, indomethacin 2.3 ± 0.6, aprotinin 1.3 ± 0.3; p > 0.1–0.3).
FIGURE 5. Indomethacin and aprotinin, given at the same time but at different sites (2 doses 14 hours apart), did not prevent a rapid decline of the BP after UC and free flow of urine (FFU; from 165 to 128 mm Hg in 30 minutes). This slope was not as rapid as those following C + UC + FFU and C + UC + ureterocaval anastomosis (UCA). Thus, a component of prostaglandin or kain or both may have played a role in the early decline of the BP. The intervals of 89' and 93' represent the average time for the acute depressor effect evoked by the second dose of aprotinin and indomethacin and the dose of captopril.

The slopes of Subgroups A and B of Group I differ from that of the indomethacin-aprotinin group (Group III, Subgroup A), and the slope of the latter group differs from that of Subgroup C of Group I (by ANOVA and LSD). These results indicate that blockade of the prostaglandin and kain systems does not prevent the kidney from exerting its antihypertensive action following unclipping. There was, however, some suppression of this function, as potentiated by captopril, by the combination of these compounds.

Group IV

The preexperiment BP in Subgroup B of Group IV (chemical papillectomy, captopril, unclipping, free flow of urine; Figure 7) was 178 ± 7 mm Hg, and the BP at the time of unclipping was 182 ± 7 mm Hg (p < 0.9). Again, there was no change in BP following unclipping.

Transient Acute Depressor Effect Evoked by a Bolus Dose of Captopril

Subgroups A and B of Group I had 24 animals (captopril plus free flow of urine or ureterocaval anas-
Tomosis). In 13 rats captopril given as a bolus evoked a short-term vasodepressor effect after a short latent period. This effect had the characteristics of the biological response following a bolus dose of ANRL. Figures 2B and 6 relate data on this effect, termed the ANRL-like effect. It is emphasized again that chemical papillectomy appeared to prevent this phenomenon. Thus, this phenomenon seemed to be mediated by the renal papilla.

Morphological Changes of the Renomedullary Interstitial Cells After Captopril and Unclipping

Captopril administration caused an outstanding change in the lipid granules of the RIC (Figure 8). These granules became more numerous and extremely large. These are the largest lipid granules encountered by this laboratory and indicate an increase in lipid levels, most likely triglycerides, in these cells. Following unclipping and the drop of the BP, the RIC degranulated as previously described, but some of the large granules remained. This change is emphasized further by the morphometric studies of the RIC (Table 1). The number of lipid granules per renomedullary interstitial cell was significantly higher ($p < 0.001$) within the clipped kidney after captopril when compared with the number in unclipped kidney after captopril administration. Other measurements were also greater within the clipped kidney as opposed to the unclipped kidney after captopril administration. These include the volume density of the RIC and their granules and the granule volume per renomedullary interstitial cell.

![Figure 8](image)

**Table 1** Morphometry of Renomedullary Interstitial Cells

<table>
<thead>
<tr>
<th>Type</th>
<th>Time (hr)</th>
<th>$n$</th>
<th>Volume density of RICG</th>
<th>Granule volume (per RIC volume)</th>
<th>Granules (per mm$^2$ of tissue section)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Captopril, chip intact</td>
<td>5</td>
<td>4</td>
<td>0.0256 ± 0.008</td>
<td>0.1895 ± 0.048</td>
<td>8.38 ± 1.37 × 10$^3$</td>
</tr>
<tr>
<td>Captopril, unclipped</td>
<td>3</td>
<td>4</td>
<td>0.0051 ± 0.0004</td>
<td>0.063 ± 0.003</td>
<td>2.67 ± 0.24 × 10$^3$</td>
</tr>
<tr>
<td>$p$ value</td>
<td></td>
<td></td>
<td>$&lt;0.02$</td>
<td>$&lt;0.02$</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

Morphometric study comparing a group receiving captopril while the chip remained intact for 5 hours and a group receiving captopril plus unclipping (blood pressure returned to normal in 17 ± 4 minutes) but followed for 3 hours. Values are means ± SEM.

RIC = renomedullary interstitial cells; RICG = granules of RIC
Antihypertensive Function of the Renal Papilla

The results summarized in Figure 9 show the antihypertensive function of the renal papilla as the rate of drop of the BP following unclipping, ΔBP/Δt (mm Hg/minute). The basic experiment consisted of unclipping plus free flow of urine with a ΔBP/Δt of 0.33. The addition of captopril tremendously potentiated this function (ΔBP/Δt of 6.22). This effect was not due to loss of sodium and volume to the outside as uretero-caval anastomosis plus captopril potentiated this function after unclipping in the same way as did the free flow of urine (ΔBP/Δt of 8.93). Aprotinin and indomethacin failed to prevent the rapid decline of the BP following captopril plus unclipping and the free flow of urine (ΔBP/Δt of 1.6), however, this slope differed from both that of the basic experiment (faster) and that of captopril and unclipping (slower). The slopes for the effect of either indomethacin alone or aprotinin alone, plus unclipping and the free flow of urine, did not differ from that obtained when the two compounds were given together (ΔBP/Δt 2.3 ± 0.6 and 1.3 ± 0.3 respectively; p > 0.5). Saralasin was not effective in potentiating the function (same ΔBP/Δt as unclipping plus the free flow of urine). Chemical papillectomy eliminated the antihypertensive function following unclipping (ΔBP/Δt of 0.01 and 0.002), even though a brisk diuresis-natriuresis-kaliuresis occurred. The urine volume varied between 1.06 and 7.3 ml (average, 4 ml), urine sodium output between 47 and 1226 μEq (average, 381 μEq), and urine potassium between 117 and 3757 μEq (average, 693 μEq) during the 3 hours after unclipping.

Discussion

The present studies on unclipping agree with those of Swales’s group, who used the two-kidney, one-clip model. Swales’s group showed that blockade of the renin-angiotensin system (with captopril or saralasin), blockade of prostaglandin synthesis (with indomethacin), and blockade of the kinin system (with aprotinin) did not prevent the antihypertensive action of the kidney after unclipping, whereas necrosis of the renal papilla (with bromethylamine hydrobromide) prevented the full expression of the antihypertensive action. Our results with the one-kidney model suggest, in addition, a potentiation of the antihypertensive action by captopril but not by saralasin and a complete lack of this function following necrosis of the renal papilla. Moreover, indomethacin and aprotinin shifted the potentiation curve in a manner suggestive of some contribution of prostaglandin and kinin toward the initial drop of the BP. The fact that indomethacin and aprotinin given separately yielded the same results suggests that the sequence entails the accumulation of kinin due to inhibition of kininase II by captopril and the stimulation of prostaglandin synthesis by the accumulated kinin. In other words, aprotinin blocks the primary product (kinin) and indomethacin blocks the secondary product (prostaglandin). The maintenance of the low BP after unclipping (i.e., at 24 hours) appeared due to mechanisms other than kinin and prostaglandin.

A sodium-volume effect was eliminated by captopril administration and uretero-caval anastomosis (no loss of sodium and volume from the animal), which caused the potentiation. Moreover, chemical papillectomy completely prevented the antihypertensive action, while a brisk diuresis-natriuresis occurred.

Hallbeck-Norlander et al. and Russell et al. demonstrated a decrease in peripheral vascular resistance as the BP was lowered after unclipping. This finding suggested the liberation of a vasodilator by the kidney. Gothberg et al. and Thörn et al. showed that as this vasodilator was detected there was bradycardia and decreased sympathetic tone. These observations are similar to those following the i.v. injection of ANRL. As ANRL can be derived from the renal venous effluent after unclipping, it is interesting that the bolus dose of captopril often evoked an ANRL-like effect. Chemical papillectomy appeared to
prevent this phenomenon. Thus, ANRL could have been a major contributor to the antihypertensive action.

Although once considered inexplicable,\textsuperscript{23,24} the antihypertensive action of the kidney after unclipping is now being elucidated. Three different approaches, using multiple approaches, have shown that the renal papilla secretes an antihypertensive hormone following unclipping. This hormone has actions antagonistic to those of ANG II. It opposes the BP effect of sodium and volume, causes bradycardia as the BP drops, suppresses the sympathetic nervous system, and causes vasodilatation.\textsuperscript{25}

Addendum

Since this paper was submitted, we have tested the effect of one-tenth of the original dose of captopril, that is, 1 mg/kg i.v., on the antihypertensive function of the kidney after unclipping. The potentiation of this function remained; \( \frac{\Delta BP}{\Delta t} = 2.4 \pm 0.56 \) (\( n = 6 \)) vs. the basic experiment (0.33 \pm 0.04; \( p < 0.001 \)). The slope moved away from that of the 10 mg/kg dose (\( \Delta BP/\Delta t = 6.22 \pm 1.64 \)), suggesting a dose response.

References

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