PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 167, 327–332 (1981)

Mechanism of Captopril-Induced Renin Release in Conscious Rats (41173)

ERNESTO L. SCHIFFRIN,[1] JOLANTA GUTKOWSKA, AND JACQUES GENEST

Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec H2W 1R7 Canada

Abstract. The increase in plasma renin activity after angiotensin I-converting enzyme inhibition by Captopril (SQ 14225) is well documented. In a study of the mechanism of Captopril-induced renin release, conscious rats were treated with Captopril (100 mg/kg per day) in their drinking water. Plasma renin activity (PRA) rose sixfold to a plateau after 5 days. At the end of 5 days of treatment with Captopril, rats carrying Alzet osmotic minipumps which infused angiotensin II (ANG II) at 200 ng/kg per min intraperitoneally had a slightly elevated PRA, as opposed to a greatly suppressed one, in ANG II-infused controls ($P < 0.05$). Urinary prostaglandin ((PG)E_2) daily excretion was unchanged by dietary Captopril. Indomethacin (5 mg/kg) injected subcutaneously significantly reduced plasma PGE_2 after 90 min, whereas PRA in Captopril-treated rats was unchanged after indomethacin injection. Results were similar in ANG II-infused Captopril-treated rats. Propranolol (10 mg/kg) injected intraperitoneally significantly reduced PRA 45 min later, but the PRA of ANG II-infused rats was not lower after propranolol injection under Captopril treatment than in similar rats not infused with ANG II. Indomethacin did not potentiate the effects of propranolol or propranolol plus ANG II on PRA of Captopril-treated rats. In conclusion, Captopril increased renin secretion by β-adrenergically mediated activation of the sympathetic nervous system and by interruption of the short feedback loop of ANG II. PGs did not appear to be involved. Other factors may be operating, since propranolol plus ANG II did not normalize PRA.

Captopril (SQ 14225) is an orally active inhibitor of angiotensin I-converting enzyme (ACE) with potent antihypertensive properties (1). It has been demonstrated that treatment with Captopril increases renin release both in experimental animals (2, 3) and in man (4, 5). The role of the different factors involved in the regulation of renin release (for review see (6)) in the renin response evoked by Captopril is unclear. Inhibition of the short feedback loop of angiotensin II (ANG II) on renin release may be an important mechanism (3). Prostaglandins may contribute to the renin response to Captopril (7). The participation of the sympathetic nervous system in renin secretion produced by hypotensive drugs in the rat has been demonstrated (8). Reflex sympathetic activation has been suggested as part of the mechanism of rise of plasma renin activity in spontaneously hypertensive rats after treatment with Captopril (9). Prostaglandins have been implicated in sympathetically induced renin release in the rat (10). Renin secretion after treatment with saralasin, an ANG II antagonist, appears to involve inhibition of the short feedback loop of ANG II and β-adrenergic stimulation (11) as well as prostaglandins (12). It therefore seemed valuable to investigate the role of the short feedback loop of ANG II, of endogenous prostaglandins and β-adrenoceptor-mediated sympathetic nervous activity on renin release produced by Captopril in conscious rats.

Materials and Methods. *Animal experiments.* Male Sprague–Dawley rats weighing approximately 250 g were kept in individual cages and exposed to light by an automated system from 6 AM to 6 PM. Rats were fed a normal sodium rat diet (Purina, Ralston, Ind.). They were placed in individual metabolic cages for studies that involved urine collection. Urine for PGE_2 determination was collected in bottles containing 100 μl of a solution of 0.05% meclophenamic acid (Parke, Davis & Co., Brockville, Ont.) in Tris buffer (0.1 mole/liter) pH 8.4.

[1] To whom editorial correspondence and requests for reprints should be addressed at: Clinical Research Institute of Montreal, 110 Pine Ave. W. Montreal, Quebec H2W 1R7 Canada.

0037-9727/81/070327-06$01.00/0

Rats had free access to tap water. Captopril was freshly dissolved in drinking water at a concentration based on the volume of water drunk the preceding day, such that daily intake was approximately 100 mg/kg. To determine whether this mode of administration of Captopril produced persistent ACE inhibition throughout the day, another group of conscious rats bearing modified Weeks' catheters (13) in the aorta and vena cava were injected intravenously with ANG I (Beckmann) and ANG II (Hypertensin, Ciba), 25 ng in a volume of 0.1 ml of 0.9% NaCl of each, at 900 and 1700 hr before and after receiving Captopril in their drinking water.

For experiments involving ANG II infusion, rats were implanted intraperitoneally with a piece of Silastic tubing (sham infusion) or an Alzet osmotic minipump, model 2001 (Alza Corp., Palo Alto, Calif.), which infused ANG II amide (Hypertensin) at 200 ng/kg per min in 0.9% NaCl. Some rats were similarly infused with Sar^1Ile^8 ANG II (Peninsula, Palo Alto, Calif.), at 1 μg/kg per min in 0.9% NaCl.

Experiments were performed between 9:00 and 12:00 AM. On the day of an experiment, conscious rats were decapitated and blood from the trunk was collected on ice in glass tubes containing EDTA during the first 5 sec after decapitation. Blood was immediately centrifuged at 4°C, and plasma was separated and stored at −20° until assayed for plasma renin activity (PRA). For the measurement of plasma PGE_2, blood was similarly collected on EDTA and 10 μl of a solution of meclophenamic acid (0.05% in Tris buffer, 0.1 mol/l, pH 8.4). Blood was centrifuged and extracted immediately (see below).

Details of drug administration prior to decapitation in each experiment are shown in the figure legends and text. Indomethacin (Sigma Chemical Co., St. Louis, Mo.) was dissolved in ethyl alcohol:olive oil (3:7) and administered subcutaneously at a dose of 5 mg/kg 90 min before decapitation. Propranolol hydrochloride (10 mg/kg) in 0.9% NaCl was injected intraperitoneally 45 min before decapitation. Control rats and rats receiving one of the two preceding drugs were injected with the solvent corresponding to the drugs not injected, by the same route.

The effects of chronic oral Captopril and intraperitoneal ANG II on blood pressure were examined in another group of rats. Blood pressure was determined by the tail-cuff technique under light ether anesthesia, with a 1010 Grass crystal microphone and recorded on a Grass Model 7 polygraph fitted with a 7P8 preamplifier.

Biochemical determinations. PRA was measured by radioimmunoassay of ANG I generated during a 2-hr incubation of plasma at 37° and pH 6.5 (14). Plasma angiotensin II levels were measured by a modification of the technique of Oster *et al.* (15). Cross-reactivity of the antibody was 70% with the ANG C-terminal penta- and heptapeptide, 97% with the hexapeptide, and 0.27% with ANG I. Within-assay coefficient of variation was 8.5% and between-assay coefficient of variation was 7.8%. Recovery of added ANG II was 82 ± 4.9% (n = 10). A blank value of 39 ± 4.7 pg/ml (measured in plasma from rats 18 hr after bilateral nephrectomy, n = 10) was not subtracted.

Plasma aldosterone was measured by a modification of the technique of Underwood and Williams (16). Plasma and urinary PGE_2 were measured by radioimmunoassay (17) using an antibody from the Pasteur Institute (Villejuif, France, lot C 79585) after extraction with cyclohexane:ethyl acetate (1:1) at pH 3.0 and chromatography through a silicic acid gel. Cross-reactivity of this antibody is 3.2% with PGE_1, 0.2% with PGA_2, and 0.06% with $PGF_{2\alpha}$.

Statistical analysis. Results were evaluated by analysis of variance and the Newman−Keuls *a posteriori* test (18). When Bartlett's test showed that variances were not homogeneous, the analysis of variance was performed on the logarithmic transform of the results. The null hypothesis was rejected when $P < 0.05$. Results are expressed as means ± SEM.

Results. PRA rose significantly with Captopril treatment after 3 days (Fig. 1, $P < 0.05$). Values after 5 days of treatment were significantly higher than after 3 days ($P < 0.05$). A plateau was reached at this time, since PRA was no higher after a further 2 days of oral Captopril. The variance of values at 5 and 7 days was similar.

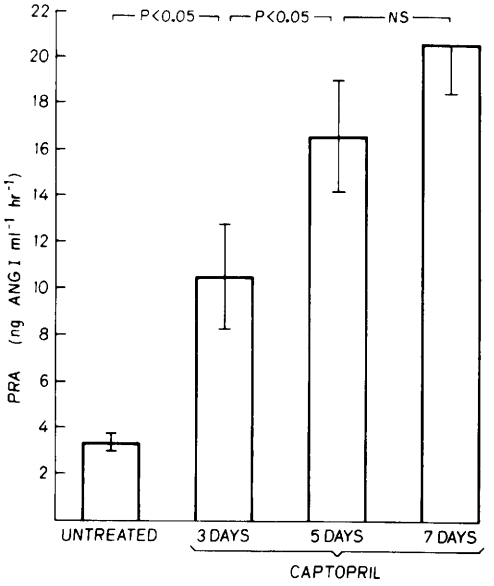

FIG. 1. Effect of oral Captopril, 100 mg/kg per day, on PRA after 3, 5, and 7 days of treatment (n = 6–8 rats per group).

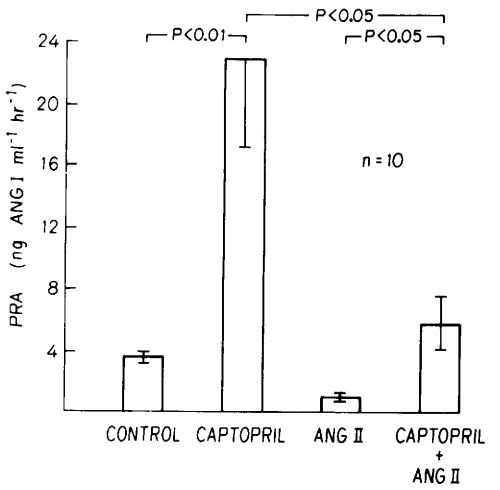

FIG. 2. Effect of chronic ANG II infusion (200 ng/kg per min) on PRA in rats receiving Captopril orally for 5 days (dose as in Fig. 1).

All subsequent studies were performed after 5 days of Captopril administration.

The effectiveness of ACE inhibition in rats treated with Captopril was demonstrated in another group of rats bearing chronic Weeks' catheters, by a 90% inhibition of the pressor response to 25 ng of ANG I injected intravenously at 9:00 AM and 5:00 PM after receiving Captopril in their drinking water on the previous day at a dose of 100 mg/kg. ANG II (25 ng intravenously) produced an identical response before and after Captopril. This showed that although rats drink water mainly during the night, ACE inhibition produced by 100 mg/kg of Captopril in their drinking fluid persists throughout the day.

When ANG II infusion was superimposed on the oral administration of Captopril, PRA was significantly lower ($P < 0.05$) than in sham-infused, Captopril-treated rats (Fig. 2). PRA was significantly higher ($P < 0.05$) than in ANG II-infused rats not receiving Captopril, which had a greatly suppressed PRA. ANG II concentration in plasma at the end of the experiment was 58 ± 6 pg/ml in control rats, 40 ± 2 pg/ml in Captopril-treated rats (equivalent to zero, since this value is the blank value of this method), 86 ± 10 pg/ml in ANG II-infused rats, and 91 ± 9 pg/ml in ANG II-infused rats treated with Captopril ($P < 0.01$ vs control, NS difference vs ANG II-infused rats not treated with Captopril). Plasma aldosterone in Captopril-treated rats was 55 ± 10 pg/ml, while in controls it was 138 ± 40 pg/ml ($P < 0.05$). Systolic blood pressure measured by the tail-cuff technique in similarly treated rats is depicted in Fig. 3. Blood pressure rose slowly under ANG II infusion, and significant differences could

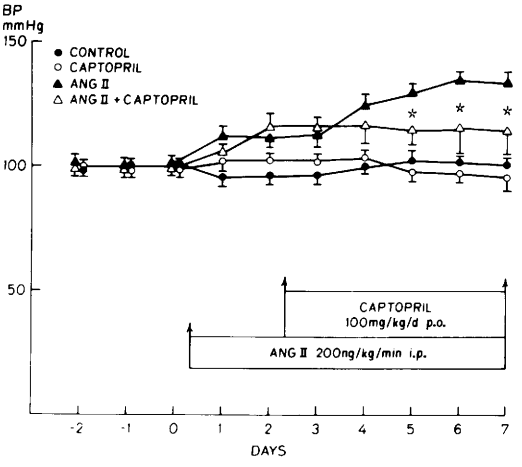

FIG. 3. Effect of chronic ANG II infusion on systolic blood pressure recorded by the tail-cuff method in rats receiving Captopril orally (doses as in Fig. 2). *P < 0.05 between both ANG II infused groups (n = 5 rats per group)

be found when comparing both groups of ANG II-infused rats with both groups of sham-infused rats. Blood pressure in Captopril-treated rats infused with ANG II was significantly lower than that of ANG II-infused rats not receiving Captopril ($P < 0.05$).

Daily urinary immunoreactive PGE_2 was measured in seven rats kept in metabolic cages while receiving Captopril in their drinking water (Fig. 4). No significant change in urinary PGE_2 excretion could be detected during the period of treatment.

Rats were injected with indomethacin 5 mg/kg subcutaneously 90 min before decapitation. Plasma immunoreactive PGE_2 was significantly lower ($P < 0.001$) after indomethacin treatment (12.5 ± 0.7 pg/ml vs 110.3 ± 24.7 pg/ml in control rats). Thus this dose of indomethacin significantly inhibited prostaglandin synthetase. PRA of indomethacin-injected rats receiving oral Captopril was not different from that of sham-injected Captopril-treated rats (Fig. 5).

The effects of the β-adrenoceptor blocker propranolol on the renin release provoked by Captopril are also illustrated in Fig. 5. Propranolol significantly reduced PRA in Captopril-treated conscious rats. This dose of propranolol reduced heart rate by 10% and did not affect blood pressure in conscious rats bearing aortic and vena cava Weeks' catheters.

Various combinations of these drugs were also tried (Fig. 5). Indomethacin did not potentiate the effects of propranolol or of the chronic infusion of ANG II. Neither

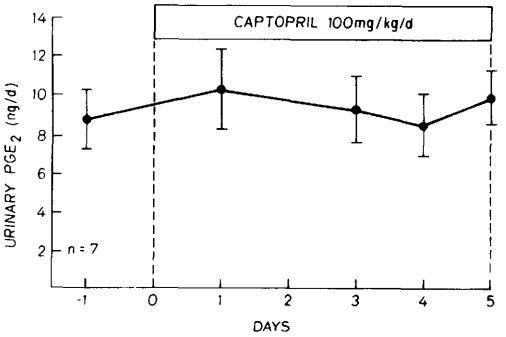

FIG. 4. Effect of oral Captopril on daily urinary PGE_2 excretion in seven conscious rats.

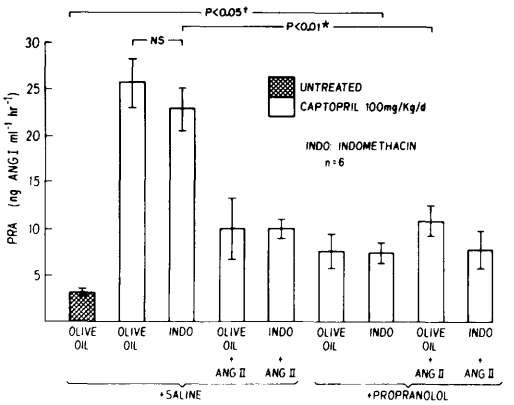

FIG. 5. Effect of indomethacin (5 mg/kg subcutaneously 90 min before decapitation), propranolol (10 mg/kg intraperitoneally 45 min before), and the corresponding solvents, in Captopril-treated rats, chronically infused or not with ANG II (200 ng/kg per min) via an intraperitoneal Alzet osmotic minipump. *$P < 0.01$—INDO vs results smaller than olive oil + ANG II. †$P < 0.05$—Captopril untreated vs results larger than INDO + propranolol.

was the PRA of Captopril-treated rats chronically infused with ANG II and injected with propranolol lower than that of rats injected only with propranolol. When all three agents were combined, PRA was not further reduced.

In order to evaluate further the role of the inhibition of the short feedback loop of ANG II, the PRA of rats treated with Captopril and chronically infused with the ANG II antagonist Sar^1Ile^8 ANG II (1 μg/kg/min) was examined. In this study Captopril increased PRA to 11.4 ± 3.5 ng $ml^{-1}hr^{-1}$ ($n = 4$). PRA in rats infused with Sar^1Ile^8 ANG II was 11.9 ± 6.3 ng $ml^{-1}hr^{-1}$ ($n = 7$), not significantly different.

Discussion. These results suggest that ANG I-converting enzyme inhibition produced by oral administration of Captopril to conscious rats increased renin secretion through two mechanisms: (i) interruption of the inhibitory short feedback loop of ANG II on renin release by juxtaglomerular cells and (ii) β-adrenoceptor activation. At the same time there was no evidence of increased endogenous prostaglandins or a role for these in Captopril-induced renin secretion.

The effect of interrupting the inhibitory short feedback loop of ANG II (19, 20) was

shown by the partial blockage of Captopril-induced renin release when rats were chronically infused with ANG II. Levels did not, however, return to normal, much less to the suppressed levels of chronically infused rats not receiving Captopril. This indicated that additional factors were involved.

We have not observed any increase in urinary daily excretion of immunoreactive PGE_2 in Captopril-treated rats. Furthermore, indomethacin was ineffective in lowering PRA in spite of the fact that it reduced plasma PGE_2 significantly, so that the dose used did adequately inhibit prostaglandin synthetase. We have previously been able to lower elevated PRA of sodium-depleted conscious rats with similar indomethacin treatment (21). In the latter situation, others have also shown increased prostaglandin production (22) and PRA decrease by indomethacin treatment (23). These results suggest that the absence of inhibitory response to indomethacin was caused by the lack of involvement of endogenous prostaglandins in Captopril-induced renin release, in agreement with our finding of unchanged urinary PGE_2 under Captopril treatment.

β-Adrenoceptor blockade was effective in reducing Captopril-stimulated renin secretion, indicating a sympathetic nervous system component in the response to Captopril. This finding is in agreement with that observed for other hypotensive drugs (8) and the angiotensin antagonist saralasin (12). In contrast to these investigators, who concluded that a prostaglandin component influences sympathetically mediated renin release, we have found evidence of the latter without prostaglandin involvement. This result agrees with our previous findings in the sodium-depleted rat (21), and those of other investigators in the sodium-depleted dog (23), suggesting that β-adrenergically mediated and prostaglandin-mediated renin secretion are independent and parallel.

When angiotensin II infusion, indomethacin and propranolol treatment were given in different combinations to Captopril-treated rats, PRA was always significantly higher than normal. This finding suggests that mechanisms other than the sympathetic nervous system and the short feedback loop of ANG II are involved in the effects of Captopril on renin release. Since the combination of ANG II infusion and propranolol treatment were no more effective in lowering PRA than either drug alone, it may be speculated that the ANG receptor mediating this action in the kidney and the β-adrenoceptor are in a close functional relationship. The other mechanism does not appear to be prostaglandin-mediated, since we find no evidence for involvement of endogenous prostaglandins in the renin response to ANG I-converting enzyme inhibition. The nature of the other mechanisms involved is not clear. Captopril may increase kinin levels by inhibiting kininase II, but there is no evidence that such an effect stimulates renin release (24).

It seems unlikely that our results might be affected by hemodynamic changes, since it has been shown that acute administration of indomethacin does not produce significant changes in heart rate or blood pressure (12). Propranolol under these conditions reduced heart rate but did not alter blood pressure in conscious rats.

Finally, we have found that an angiotensin II antagonist, Sar^1Ile^8 ANG II, did not further increase renin release. This suggests that the mechanisms involved in renin secretion by ANG II antagonism and ANG I-converting enzyme inhibition are similar, i.e., β-adrenergic stimulation and interruption of the short feedback loop of ANG II.

Another observation of interest in our study has been the finding that the slowly developing pressor effect found after ANG II infusion (only 10–15% is available systemically after intraperitoneal infusion, according to the plasma concentration of ANG II we have measured) is blunted in rats treated with Captopril. This suggests a role for factors other than renin in the hypotensive action of Captopril, as previously concluded from acute experiments in rats (25) and dogs (26).

The authors acknowledge the expert technical assistance of M. Chamberland and M. Deslongchamps and the secretarial aid of F. De Coste in typing this manuscript. We are grateful to Dr. Z. P. Horowitz of

the Squibb Institute for Medical Research for the supply of Captopril (SQ 14225) used in this study. This work was supported by a group grant given by the Medical Research Council of Canada to the Multidisciplinary Group on Hypertension of the Clinical Research Institute of Montreal and by a grant from the Canadian Heart Foundation.

1. Ondetti, M. A., Rubin, B., and Cushman, D. W., Science **196**, 441 (1977).
2. Bengis, R. G., Coleman, T. G., Young, D. B., and McCaa, R. E., Circ. Res. **43**, (Suppl. I), I-45 (1978).
3. McCaa, R. E., Hall, J. E., and McCaa, C. S., Circ. Res. **43** (Suppl. I), I-32 (1978).
4. Brunner, H. R., Gavras, H., Waeber, B., Kershaw, G. R., Turini, G. W., Vukovich, R. A., McKinstry, D. N., and Gavras, I., Ann. Intern. Med. **90**, 19 (1979).
5. Larochelle, P., Genest, J., Kuchel, O., Boucher, R., Gutkowska, J., and McKinstry, D., Canad. Med. Assoc. J. **121**, 309 (1979).
6. Davis, J. O., and Freeman, R. H., Physiol. Rev. **56**, 1 (1976).
7. Abe, K., Itoh, T., Satoh, M., Haruyama, T., Imai, Y., Goto, T., Satoh, K., Otsuka, Y., and Yoshinaga, K., Life Sci. **26**, 561 (1979).
8. Keeton, T. K., and Pettiger, W. A., J. Pharmacol. Exp. Ther. **208**, 303 (1979).
9. Antonaccio, M. J., Harris, D., Goldenberg, H., High, J. P., and Rubin, B. Proc. Soc. Exp. Biol. Med. **162**, 429 (1979).
10. Campbell, W. B., Graham, R. M., and Jackson, E. K., J. Clin. Invest. **64**, 448 (1979).
11. Keeton, T. K., Pettinger, W. A., and Campbell, W. B., Circ. Res. **38**, 531 (1976).
12. Campbell, W. B., Jackson, E. K., and Graham, R. M., Hypertension **1**, 637 (1979).
13. Weeks, J. R., and Jones, J. A., Proc. Soc. Exp. Biol. Med. **104**, 646 (1960).
14. Gutkowska, J., Boucher, R., and Genest, J., Union Med. Canad. **106**, 446 (1977).
15. Oster, P., Hackenthal, E., and Hepp, R., Experientia **29**, 353 (1973).
16. Underwood, R., and Williams, G. H., J. Lab. Clin. Med. **79**, 848 (1972).
17. Jaffe, B. M., Behrman, H. R., and Parker, C. W., J. Clin. Invest. **52**, 398 (1973).
18. Winer, B. J., "Statistical Principles in Experimental Design." McGraw-Hill, New York (1971).
19. Shade, R. E., Davis, J. O., Johnson, J. A., Gottshall, R. W., and Spielman, W. S., Amer. J. Physiol. **224**, 926 (1973).
20. Naftilan, A. J., and Oparil, S., Amer. J. Physiol. **235**(1), F62 (1978).
21. Schiffrin, E. L., Garcia, R., Gutkowska, J., Boucher, R., and Genest, J., Proc. Soc. Exp. Biol. Med. **165**, 151 (1980).
22. Stahl, R. A. K., Attallah, A. A., Bloch, D. L., and Lee, J. B., Amer. J. Physiol. **237**(5), F344 (1979).
23. De Forrest, J. M., Davis, J. O., Freeman, R. H., Seymour, A. A., Rowe, B. P., Williams, G. M., and Davis, T. P., Circ. Res. **47**, 99 (1980).
24. Osborn, J. L., Noordewier, B., Hook, J. B., and Bailie, M. D., Proc. Soc. Exp. Biol. Med. **159**, 249 (1978).
25. Marks, E. S., Bing, R. F., Thurston, H., and Swales, J. D., Clin. Sci. **58**, 1 (1980).
26. Tree, M., and Morton, J. J., Clin. Sci. **59**, 451 (1980).

Received March 3, 1981 P.S.E.B.M. 1981, Vol 167.