PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 162, 429–433 (1979)

The Effects of Captopril, Propranolol, and Indomethacin on Blood Pressure and Plasma Renin Activity in Spontaneously Hypertensive and Normotensive Rats (40697)

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The mechanisms by which renin release may be altered have been the subject of numerous reports and have been reviewed by several authors (1–5). In the past, most studies centered around the control of renin release by the intrarenal receptors in the afferent arteriole and macula densa, adrenergic receptors, and a few humoral agents, such as angiotensin II and antidiuretic hormone. However, it has become obvious in recent years that prostaglandins may also play an important role in controlling renin release. Prostaglandins such as PGE_1, PGE_2, PGA_1, and prostacyclin (PGI_2) cause renin release both *in vitro* and *in vivo* (6–10). Similarly, the precursors to the stable prostaglandins, arachidonic acid, and the prostaglandin endoperoxides also cause an increase in renin release (11–14). Moreover, inhibition of prostaglandin synthesis reduces resting plasma renin activity (PRA) and prevents increases in renin secretion caused by arachidonic acid as well as the increase caused by changes in posture and the diuretic furosemide (15–22).

Captopril (SQ 14,225, D-3-mercapto-2-methylpropanoyl-L-proline) is an orally effective inhibitor of angiotensin-converting enzyme which reduces blood pressure in normal renin as well as high renin animal models of hypertension (23–28). Similarly, captopril is an effective antihypertensive agent in low, normal, and high renin essential hypertension as well as in renal hypertension in man (29–31). In all of the studies in which it was measured, PRA increased substantially after captopril administration in both normal and hypertensive animals and man (24, 25, 29–32). The mechanism(s) by which captopril raises plasma renin activity has not been examined although many possibilities such as reflex sympathetic activation, interruption of a negative feedback system, and increased renal prostaglandin formation could very well play a role.

The purpose of this study was to examine the effects of captopril alone and in combination with either the β-receptor antagonist, propranolol, or the cyclooxygenase inhibitor, indomethacin, on blood pressure and PRA in normotensive rats and in spontaneously hypertensive rats.

Materials and methods. Ten- to fourteen-week-old male normotensive rats of the Wistar–Kyoto strain (WKY) and spontaneously hypertensive rats of the Okamoto and Aoki strain (SHR) were obtained from Taconic Farms, Germantown, N.Y. and were placed on a normal rat chow diet with water *ad libitum*. All drugs were administered by gavage.

Captopril was synthesized at the Squibb Institute for Medical Research. Indomethacin was kindly donated by Merck, Sharpe and Dohme, propranolol HCl was kindly donated by Ayerst Laboratories, and arachidonic acid was purchased from Sigma Chemical Company.

Statistics were calculated using an unpaired Student's t test. A value of $P < 0.05$ was considered to be significant.

Blood pressure measurements. Approximately 18 days before each of the dosage intervals described below, indwelling abdominal aortic cannulae were implanted in rats anesthetized with pentobarbital sodium and, after the 18-day recovery period, direct mean arterial blood pressures in conscious rats were recorded by the method of Laffan *et al.* (25).

Plasma renin activity. On the day of blood pressure measurement, 1 hr after any drug treatment, 0.5 ml of blood from the implanted aortic cannula of all rats was collected into a tube containing 50 μl of 0.25 m*M* EDTA, pH 7. Plasma was prepared by centrifuging the samples at 2500 g for 30 min at 4°. Two hundred microliters of plasma was placed in a 400-μl polypropylene tube, tightly capped, and stored in an ultra-low temperature

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freezer (−70°) until analyzed. PRA values were usually determined within 3 weeks of storage.

PRA values, expressed in terms of nanograms of Angiotensin I (Asp^1–Ile^5–angiotensin I) generated per milliliter of plasma per hour of incubation at 37°, were determined using the Squibb Angiotensin I Immunotope Kit. The pH of the plasma was adjusted to 6.5 which is the pH optimum for PRA in rats.

Results. Effects of captopril, indomethacin, and propranolol on mean blood pressure (MBP). Treatment of spontaneously hypertensive rats (SHR) with captopril (30 mg/kg p.o. daily for 3 days) resulted in a significant reduction in MBP (Table I). Similar, though smaller, reductions in MBP were observed after propranolol (30 mg/kg p.o. daily for 3 days). The combination of captopril and propranolol administered together also resulted in significant reductions in MBP which were not significantly different from those observed with either captopril or propranolol given alone (Table I). Propranolol treatment resulted in complete blockade of the normally observed tachycardia (54.3 ± 2.6 beats/min) caused by isoproterenol administration, 1 μg/kg i.v.

TABLE I. EFFECTS OF CAPTOPRIL AND PROPRANOLOL (BOTH AT 30 mg/kg p.o. DAILY FOR 3 DAYS), ALONE AND IN COMBINATION, ON MEAN BLOOD PRESSURE (MBP, mm Hg)[a] OF SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND NORMOTENSIVE RATS (NTR)

	Control MBP	N	Captopril	Propranolol	Captopril + propranolol
SHR	183.6 ±3.4	7	136.7 ±6.5 $P < 0.001$		
	188.0 ±3.0	10		153.6 ±5.6 $P < 0.001$	
	178.0 ±2.3	7			150.6 ±7.7 $P < 0.005$
NTR	129.5 ±3.4	10	110.3 ±5.1 $P < 0.02$		
	130.7 ±2.1	6		105.8 ±7.0 $P < 0.01$	
	128.6 ±2.8	7			108.0 ±5.3 $P < 0.02$

[a] Values shown after drugs are the maximum changes observed over a 3-hr observation period on the third day of dosing.

TABLE II. EFFECTS OF CAPTOPRIL (30 mg/kg p.o DAILY FOR 3 DAYS) AND INDOMETHACIN (2.5 mg/kg p.o. DAILY FOR 3 DAYS), ALONE AND IN COMBINATION, ON MEAN BLOOD PRESSURE (MBP, mm Hg)[a] OF SPONTANEOUSLY HYPERTENSIVE (SHR) AND NORMOTENSIVE RATS (NTR)

	Control MBP	N	Captopril	Indomethacin	Captopril + indomethacin
SHR	169.0 ±2.0	10	145.3 ±4.0 $P < 0.001$		
	175.9 ±3.6	9		162.7 ±6.7	
	183.4 ±5.1	10			138.6 ±5.4 $P < 0.01$
NTR	118.9 ±2.1	9	110.7 ±4.3 $P < 0.05$		
	122.4 ±1.5	10		117.8 ±2.9	
	116.5 ±1.7	4			100.8 ±2.5 $P < 0.05$

[a] Values shown after drugs are the maximum changes observed over a 3-hr observation period on the third day of dosing.

Qualitatively similar results were observed in normotensive rats (Table I).

In other SHR, captopril (30 mg/kg p.o. daily) administered for 3 days once again caused a significant reduction in MBP (Table II). Treatment with indomethacin (2.5 mg/kg p.o. daily for 3 days) had no effect on MBP by itself nor did it affect the reduction in MBP caused by captopril (Table II).

Once again, qualitatively similar results were observed in normotensive rats (Table II).

Effects of captopril, indomethacin, and propranolol on plasma renin activity (PRA). PRA of untreated SHR were significantly lower than those of NTR (Fig. 1). Administration of captopril (30 mg/kg p.o. daily for 3 days) caused significant, marked elevation of PRA in both SHR and NTR. Although the maximum absolute PRA values after captopril in SHR and NTR were not significantly different from each other (Fig. 1), the mean value of the differences was (48.03 vs 22.7 for SHR and NTR, respectively; $P < 0.05$).

Indomethacin (2.5 mg/kg p.o. daily for 3 days) significantly reduced PRA in both SHR and NTR (Fig. 2). Whereas indomethacin had no significant effect on changes in PRA caused by captopril in SHR, it caused a mod-

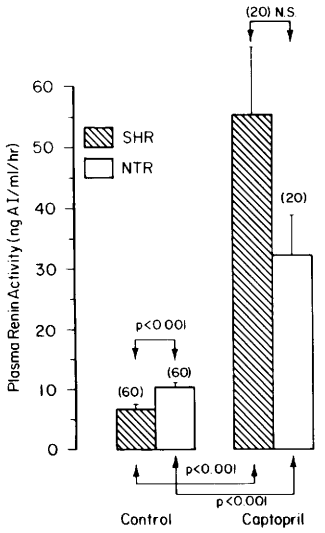

FIG. 1. Plasma renin activity (ng angiotensin I/ml/hr) in conscious spontaneously hypertensive (SHR) and Wistar–Kyoto normotensive (NTR) rats before and after captopril (30 mg/kg p.o.). Figures in parentheses indicate numbers of rats/group.

est, though insignificant, inhibition of renin release in NTR (Fig. 2, cf. Fig. 1). The dose of indomethacin used caused a 61.1% inhibition of the depressor response (64.2 ± 9.8 mm vs 25.0 ± 5.5 mmHg, $P < 0.001$) to arachidonic acid (0.3 mg/kg i.v.) in other SHR. Higher doses of indomethacin were found to be toxic.

Propranolol (30 mg/kg p.o. daily for 3 days) reduced PRA in both SHR and NTR (Fig. 3). Propranolol also prevented any significant rise in PRA in SHR dosed with captopril whereas the effect in NTR was not significantly reduced (Fig. 3, cf. Fig. 1).

Discussion. The present study demonstrates, as have others (32–34), that adult SHR have significantly lower plasma renin activities (PRA) than their normotensive genetic counterparts. We have also confirmed the ability of captopril to increase plasma renin activity and to decrease blood pressure of SHR (25) and have further demonstrated an increase of PRA in WKY–NTR rats after captopril, which is similar to that of the Sprague–Dawley strain (26).

Both indomethacin and propranolol caused significant reductions in plasma renin activity in both SHR and NTR before the administration of captopril suggesting that renin release in these animals is under tonic control by both β-receptors and prostaglandins as has been previously suggested (see Introduction for references). In SHR, propranolol markedly decreased, whereas indomethacin was without effect on, the increase in PRA caused by captopril administration. However, in NTR, neither propranolol nor indomethacin significantly inhibited the effects of captopril on PRA. These results sug-

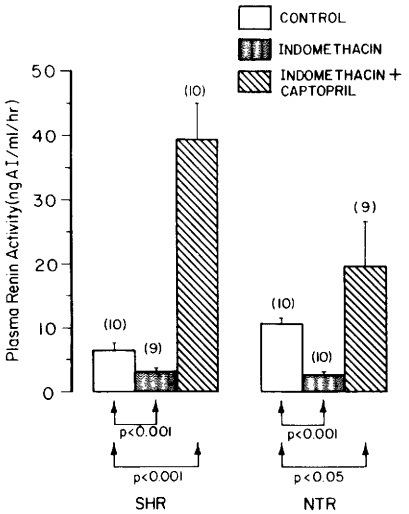

FIG. 2. Plasma renin activity (ng angiotensin I/ml/hr) in conscious SHR and NTR before and after either indomethacin alone (2.5 mg/kg p.o.) or indomethacin + captopril (30 mg/kg p.o.). Figures in parentheses indicate numbers of rats/group.

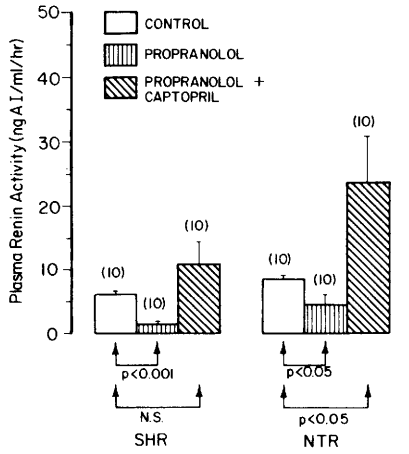

FIG. 3. Plasma renin activity (ng angiotensin I/ml/hr) in conscious SHR and NTR before and after either propranolol (30 mg/kg p.o.) or propranolol + captopril (30 mg/kg p.o.). Figures in parentheses indicate numbers of rats/group.

gest that, at least in SHR, the increase in PRA caused by captopril administration is mediated mainly by activation of renal β-receptors. Furthermore, since propranolol had little effect on either blood pressure or the increase in PRA in NTR, this activation was probably a result of a compensatory reflex sympathetic activation caused by the reduction in blood pressure by captopril. It should be noted that propranolol also prevents the increase in plasma renin activity caused by agents such as saralasin which act by interrupting the short-loop intrarenal feedback mechanism (35). Thus, it is conceivable that propranolol acts distal to the angiotensin II receptor in this short loop mechanism after captopril administration which reduces angiotensin II formation.

Interestingly, the addition of propranolol to captopril therapy in SHR had no significant effect on the reduction in blood pressure by captopril indicating the high plasma renin activity and angiotensin I level did not substantially override the inhibition of the angiotensin-converting enzyme in these animals.

Indomethacin had no significant inhibitory effect on either the antihypertensive action of captopril in SHR or on the increase in plasma renin activity in both SHR and NTR. Thus, prostaglandin formation does not seem to play a significant role in the ability of captopril to decrease blood pressure in SHR or in raising renin levels. However, indomethacin did significantly decrease resting PRA values in both SHR and NTR suggesting that (i) the dose of indomethacin used in this study was sufficient to at least partially block prostaglandin formation (further demonstrated by the significant inhibition of the cardiovascular effect of arachidonic acid) and (ii) prostaglandins play a role in modulating renin release under normal circumstances as suggested by others (see Introduction). It is possible that higher doses of indomethacin could have had more profound inhibitory effects on PRA changes caused by captopril. This is unlikely in SHR since propranolol caused a virtual abolition of the PRA response, but this cannot be ruled out in NTR. Higher doses of indomethacin were found to be toxic.

Summary. Captopril reduced blood pressure and increased PRA in both SHR and NTR. Both propranolol and indomethacin caused significant reductions in resting PRA of both SHR and NTR but only propranolol caused an inhibition of the PRA changes caused by captopril. Furthermore, since propranolol was highly effective only in SHR and captopril decreased blood pressure much more in SHR than NTR, the increase in PRA caused by captopril in SHR was probably reflexly mediated by compensatory sympathetic activation, all of which actions were a consequence of reducing angiotensin II formation.

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Received May 11, 1979. P.S.E.B.M. 1979, Vol. 162.