

Failure of Indomethacin to Inhibit Saralasin-Stimulated Plasma Renin Activity in Conscious Dogs with Mild Sodium Depletion (41216)

J. R. DIETZ, J. O. DAVIS, R. H. FREEMAN, S. F. ECHTENKAMP, AND D. VILLARREAL¹

Department of Physiology, University of Missouri, School of Medicine, Columbia, Missouri 65212

Abstract. The hypothesis that prostaglandins play a role in the angiotensin II control mechanism for renin release was examined by interrupting the short-loop negative feedback of angiotensin II on the JG cells with the competitive angiotensin II antagonist saralasin. Mildly sodium-depleted conscious dogs received either indomethacin, or the vehicle alone followed by an intravenous infusion of saralasin. Saralasin produced equivalent increases in PRA in the vehicle- and indomethacin-treated groups with no detectable change in arterial pressure. Plasma renin activity increased in the indomethacin-treated group despite a 58% fall in the rate of excretion of PGE₂. These results suggest that in the conscious dog renal prostaglandins do not play an appreciable role in angiotensin II feedback control of renin release.

Renin secretion is influenced by a short-loop negative feedback mechanism whereby angiotensin II acts directly on the juxtaglomerular cells to decrease renin release (1). The inhibitory action of angiotensin II on renin release has been demonstrated *in vitro* (2) and also in intact animal preparations during intrarenal infusion of the peptide (3-5).

Since prostaglandins increase renin release (6, 7), a number of studies have focused on the role of renal prostaglandins as an intermediary messenger in angiotensin feedback control of renin secretion. It is well known that inhibition of angiotensin II receptors with the competitive antagonist saralasin or preventing angiotensin II formation with a converting enzyme inhibitor stimulates renin secretion but there is little agreement as to the effect of inhibitors of prostaglandin synthesis on this response (8-11). The present study examines this question in conscious dogs with mild sodium depletion by interrupting the short feedback loop with saralasin in the presence and absence of the prostaglandin synthetase inhibitor indomethacin.

Methods. The experiments were conducted in five female mongrel dogs weigh-

ing between 15.5 and 22.5 kg (\bar{X} = 18.7 kg). They were housed in individual metabolism cages to measure daily electrolyte balances. Under sodium pentobarbital anesthesia chronic indwelling catheters were placed in the femoral vessels and passed under the skin to exit at the back of the neck. The dogs were allowed to recover for at least 1 week during which time they were trained to lie on a padded table and control measurements for plasma renin activity (PRA) were made. Four days prior to the acute experiments the animals received an injection of 0.5 ml of Mercurhydrin and sodium intake was reduced from a normal intake of 60 meq/day to <3 meq/day. This regimen of mild sodium depletion resulted in a negative sodium balance of 66 ± 6 meq (five dogs) at the time of the acute indomethacin experiments and a negative sodium balance of 59 ± 5 meq prior to the acute experiments when the same five dogs received the vehicle only.

All experiments were conducted in the conscious state with the dogs resting on a padded table. A Foley catheter was placed in the bladder for collecting urine and arterial blood pressure and heart rate were monitored continuously through the femoral artery catheter by use of a Satham pressure transducer (Model P23Db).

One hour prior to the start of the acute study, a priming solution of *p*-aminohip-

¹ Recipient of a research fellowship from the Missouri Heart Association.

TABLE I. THE EFFECTS OF VEHICLE ALONE (V) OR INDOMETHACIN (I) AND SUBSEQUENT ANGIOTENSIN II BLOCKADE ON PRA, ARTERIAL PRESSURE AND RENAL FUNCTION IN FIVE CONSCIOUS, SODIUM-DEPLETED DOGS

		Indomethacin (5 mg/kg) or vehicle alone		Saralasin 6 μg/kg/min						
		C ₁ ^a	C ₂	C ₃	E ₁	E ₂	E ₃	R ₁	R ₂	R ₃
PRA (ngAl/ml/hr)	V	2.2 ± 0.3 ^b	2.3 ± 0.3	2.9 ± 0.7	2.3 ± 0.6	3.7 ± 0.6	5.5 ± 1.6*	5.1 ± 0.9*	4.5 ± 0.7	3.3 ± 0.6
	I	1.9 ± 0.4	1.6 ± 0.3	2.9 ± 1.3	3.1 ± 1.5	5.8 ± 1.2*	7.4 ± 1.3**	5.9 ± 1.3*	4.6 ± 1.0	4.2 ± 1.1
MAP (mmHg)	V	95 ± 2	95 ± 3	99 ± 3	93 ± 7	97 ± 3	98 ± 3	99 ± 2	100 ± 2	98 ± 3
	I	102 ± 2	103 ± 2	101 ± 4	95 ± 3	97 ± 4	96 ± 4	101 ± 3	96 ± 3	101 ± 2
HR (beats/min)	V	84 ± 3	82 ± 2	88 ± 3	87 ± 6	86 ± 5	88 ± 4	87 ± 4	84 ± 2	87 ± 3
	I	85 ± 4	84 ± 4	77 ± 5	81 ± 5	81 ± 6	80 ± 4	79 ± 4	81 ± 3	83 ± 6
UF (ml/min)	V	0.37 ± .02	0.46 ± 0.07	0.81 ± 0.17	0.68 ± 0.12	0.51 ± 0.08	0.47 ± 0.01	0.48 ± 0.06	0.43 ± 0.07	0.41 ± 0.07
	I	0.29 ± .05	0.31 ± 0.06	0.27 ± 0.05	0.17 ± 0.06	0.20 ± 0.05	0.22 ± 0.01	0.28 ± 0.05	0.29 ± 0.05	0.30 ± 0.05
C _{Cr} (ml/min)	V	68 ± 3	64 ± 6	64 ± 2	79 ± 3	67 ± 6	86 ± 7*	91 ± 5*	84 ± 5	80 ± 6
	I	66 ± 7	63 ± 6	65 ± 8	58 ± 13	60 ± 11	62 ± 15	62 ± 8	62 ± 8	69 ± 7
C _{PAH} (ml/min)	V	211 ± 20	227 ± 23	240 ± 23	229 ± 22	229 ± 22	251 ± 38	265 ± 33	265 ± 30	237 ± 24
	I	209 ± 19	196 ± 21	183 ± 29	180 ± 48	203 ± 67	219 ± 54	207 ± 44	208 ± 34	229 ± 41
U _{Na} V (μEq/min)	V	5.5 ± 2.1	5.0 ± 1.5	22.6 ± 7.8	29.7 ± 13.0*	16.4 ± 7.0	9.0 ± 2.4	9.6 ± 3.5	17.5 ± 11.5	16.4 ± 10.8
	I	1.9 ± 0.8	2.5 ± 0.8	2.1 ± 1.2	1.8 ± 0.7	2.4 ± 1.3	2.8 ± 1.3	1.6 ± .6	2.1 ± 0.8	2.8 ± 0.9

Note. PRA, plasma renin activity; MAP, mean arterial pressure; HR, heart rate; UF, urine flow; C_{Cr} and C_{PAH}, clearance of creatinine and p-aminohippurate, respectively; U_{Na}V = rate of excretion of sodium.
^a Periods are each 30 min in length.
^b Mean ± SEM
 * P < 0.05 from corresponding C₁ & C₂.
 ** P < 0.01 from corresponding C₁ & C₂.

purate (PAH), 10 mg/kg, and creatinine (Cr), 50 mg/kg, was administered intravenously followed immediately by a continuous intravenous infusion of PAH and Cr at 4.3 and 21.6 mg/min, respectively, at a rate of 0.3 ml/min.

The experiments consisted of nine 30-min clearance periods. Following two control periods the animals received either an intravenous injection of indomethacin (5 mg/kg) dissolved in 5 ml of ethanol and 5 ml of 0.5 M phosphate buffer or the vehicle alone. Thirty minutes after the injection each animal received a 90-min intravenous infusion of saralasin (1-Sar-8-Ala-angiotensin II, Norwich) at a rate of 6 μ g/kg/min. The final three 30-min periods served as a recovery. Saralasin completely abolished the pressor response to intravenous bolus injections of 1 and 2 μ g of angiotensin II.

Plasma and urine PAH and Cr concentrations and urine electrolyte concentrations were determined by standard techniques. PRA was measured by radioimmunoassay for angiotensin I as described by Sealey *et al.*, (12). Urinary prostaglandin E_2 (PGE_2) content was measured according to the method of Dray *et al.*, (13).

The results were compared by an analysis of variance and Newman-Keuls test or by paired *t* test for comparison within groups. A *P* value of <0.05 was considered significant.

Results. The results are summarized in Table I. In five mildly sodium-depleted dogs treated with the vehicle alone a 90-min saralasin infusion (6 μ g/kg/min) produced a significant increase in PRA from 2.3 ± 0.3 to 5.5 ± 1.6 (E_3) ngAI/ml/hr ($P < 0.05$). PRA remained elevated during the first recovery period (R_1) and then returned toward the control value. No detectable change in mean arterial pressure (MAP), heart rate (HR), or urine flow was observed. C_{Cr} was significantly increased by 30% from control values during the final period of the saralasin infusion (E_3) and by 40% during the first recovery period (R_1) (both $P < 0.05$); C_{PAH} failed to change. The rate of excretion of sodium ($U_{Na}\dot{V}$) was significantly elevated from control only during the first period of saralasin infusion (E_1 , $P < 0.05$). When the same five sodium-

depleted dogs were treated with an intravenous injection of indomethacin (5 mg/kg), saralasin produced an increase in PRA from 1.8 ± 0.4 to 5.8 ± 1.2 ngAI/ml/hr during the second period of infusion (E_2 , $P < 0.05$) and to 7.4 ± 1.3 ngAI/ml/hr during the third period of infusion (E_3 , $P < 0.01$). MAP and HR were not altered by indomethacin or the infusion of saralasin. In addition no detectable changes in renal function were observed with saralasin infusion in the indomethacin-treated animals. There were no significant differences in PRA between the two groups at any time period.

PGE_2 was measured on urine samples collected from the five indomethacin-treated dogs during the control periods (C_1 and C_2) and during the second and third periods of the saralasin infusion (E_2 and E_3). These results are shown in Fig. 1. Saralasin increased PRA in the indomethacin-treated animals despite a 58% decrease in the rate of excretion of PGE_2 from 819 ± 235 (average of C_1 and C_2) to 344 ± 108 pg/min ($P < 0.05$) at the peak of the renin response (E_3). The decrease in PGE_2 excretion occurred in the absence of a change in urine flow.

Discussion. The results show that an in-

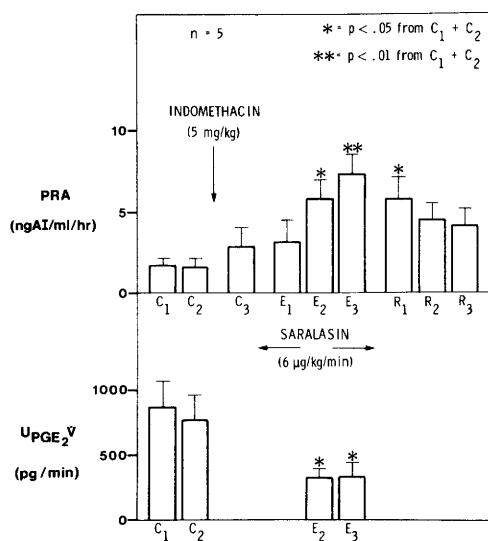


FIG. 1. The effect of indomethacin on saralasin-stimulated plasma renin activity (PRA) and the urinary excretion rate of prostaglandin E_2 (PGE_2) in conscious sodium-depleted dogs. Values are means \pm 1 SEM. Periods C_{1-3} , E_{1-3} , and R_{1-3} are each 30 min in length.

crease in PRA produced by the saralasin infusion in mildly sodium-depleted dogs was not affected by a dose of indomethacin which markedly decreased the rate of PGE₂ excretion. These findings suggest that renal prostaglandins do not play an important role in the angiotensin II negative feedback mechanism for control of renin secretion. The saralasin infusion elicited increases in PRA without a detectable fall in arterial pressure in this mildly sodium-depleted state (63 meq of sodium). This is important since a reduction in arterial pressure might stimulate renin release via the renal vascular receptor, the macula densa, or the adrenergic nervous system (1). In preliminary experiments in this laboratory it was observed that saralasin failed to alter PRA or arterial pressure in sodium-repleted dogs. In dogs with severe sodium depletion (140 meq of sodium), intravenous saralasin infusion increased PRA but also decreased arterial pressure (14). Several investigators have reported a direct relationship between basal PRA and the blood pressure reduction and the rise in PRA with intravenous saralasin infusions (15–17) and have pointed out the importance of sodium balance in this response. The present results obtained in conscious dogs with mild sodium depletion [to elevate plasma angiotensin II levels (see Methods)] are consistent with the observations that angiotensin II decreases renin secretion by a direct action on the JG cells (2, 4, 18).

The present results obtained in conscious dogs agree with those of Antonaccio *et al.* (9) who showed that in the conscious rat indomethacin did not affect captopril-stimulated PRA. Abe *et al.* (8) found that daily, oral doses of indomethacin blocked captopril-stimulated PRA in both hypertensive and normotensive patients but they failed to report changes in blood pressure or sodium balance in these patients maintained on a high salt diet. Measurements of sodium balance are important since daily, oral indomethacin administration can decrease PRA due to sodium retention (19). Campbell *et al.* (10) reported that in conscious rats subcutaneous administration of indomethacin or meclofenamate blocked

saralasin-stimulated PRA. Their results, however, are somewhat difficult to interpret since all drugs were administered subcutaneously and it is not clear whether indomethacin or meclofenamate altered the rate of absorption of saralasin. This is important since PRA was measured just 20 min after subcutaneous saralasin administration. Furthermore, it was not shown that saralasin administered by this route in the presence of the inhibitors effectively blocks the response to endogenous angiotensin II. In the present study an intravenous saralasin infusion blocked the increase in arterial pressure observed with 2 μ g injections of angiotensin II and no differences in the renin response to blockade were observed between the indomethacin- and vehicle-treated groups. Collectively, these previously reported findings of others and the present data do not support the suggestion that saralasin-stimulated PRA is mediated by prostaglandins.

1. Davis, J. O., and Freeman, R. H., *Physiol. Rev.* **56**(1), 1 (1976).
2. Michelakis, A. M., *Proc. Soc. Exp. Biol. Med.* **137**, 1106 (1971).
3. Blair-West, J. R., Coghlan, J. P., Denton, D. A., Funder, J. W., Scoggins, B. A. and Wright, R. D., *Amer. J. Physiol.* **220**, 1309 (1971).
4. Shade, R. E., Davis, J. O., Johnson, J. A., Gotshall, R. W., and Spielman, W. S., *Amer. J. Physiol.* **224**(4), 926 (1973).
5. McDonald, K. M., Tarer, S., Antoine de Torrente, G. A., and Schrier, R. W., *Amer. J. Physiol.* **228**(5), 1562 (1975).
6. Gerber, J. G., Branch, R. A., Nies, A. S., Gerkens, J. F., Shand, D. G., Hollifield, J., and Oates, J. A., *Prostaglandins* **15**, 81 (1978).
7. Seymour, A. A., and Zehr, J. E., *Circ. Res.* **45**, 13, (1979).
8. Abe, K., Itoh, T., Satoh, M., Haruyama, T., Imai, Y., Goto, T., Satoh, K., Otsuka, Y., and Yoshinga, K., *Life Sci.* **26**(7), 561 (1980).
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., Jackson, E. K., and Graham, R. M., *Hypertension* **1**, 637 (1979).
11. DeForrest, 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).
12. Sealey, J. E., Laragh, J. H., Gerten-Barnes, J.,

- and Accto, R. M., in "Hypertension Manual" p. 621. Yorke Medical/Dun-Donnelley, New York (1974).
13. Dray, F., Charbonnel, B., and Maclouf, J., *Eur. J. Clin. Invest.* **5**, 311 (1975).
 14. Stephens, G. A., Davis, J. O., Freeman, R. H., Watkins, B. E., and Khosla, M. C., *Endocrinology* **101**(2), 378 (1977).
 15. Baer, L., Parra-Carrillo, J. Z., and Radichevich, I., *Kid. Int.* **15** (Suppl. 9), S-60 (1979).
 16. Conway, J., Hatton, R., Keddie, J., and Dawes, P., *Hypertension* **1**, 402 (1979).
 17. Hollenberg, N. K., and Williams, G. H., *Kid. Int.* **15** (Suppl. 9), S-29 (1979).
 18. Vander, A. J., and Geelhoed, G. W., *Proc. Soc. Exp. Biol. Med.* **120**, 399 (1965).
 19. Frolich, J. C., *Arch. Int. Pharmacodyn. Ther. Suppl.*, 213 (1980).
-

Received March 13, 1981. P.S.E.B.M. 1981, Vol. 167.