

Effects of Indomethacin and Meclofenamate on Renin Release and Renal Hemodynamic Function during Chronic Sodium Depletion in Conscious Dogs

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SUMMARY We studied the control of renin release and renal hemodynamic function by administering prostaglandin synthetase inhibitors to conscious sodium-depleted dogs with blockade of the adrenergic nervous system induced by bilateral renal denervation and propranolol administration. Indomethacin (10 mg/kg) reduced plasma renin activity (PRA) by 59% from a high sodium-depleted value, but PRA was still 3 times the normal sodium-repleted level. Arterial pressure, C_{Cr} , C_{PAH} , urine flow, and potassium excretion fell strikingly. Similar results were obtained with meclofenamate. When SQ 14,225 was given to another group of conscious, sodium-depleted dogs with adrenergic nervous system blockade, PRA increased from the high sodium-depleted level of 5.7 to 29.3 ng of Angiotensin I (AI)/ml per hour; indomethacin (10 mg/kg) appeared to reduce PRA ($0.05 < P < 0.1$) but to only 12.1 ng of AI/ml per hour, which is 17 times the normal level. This high level of PRA after blockade of the adrenergic nervous system and injection of indomethacin suggests that important mechanisms other than norepinephrine and renal prostaglandins control renin release; it is proposed that both the renal vascular receptor and the macula densa are involved. The marked decreases in C_{Cr} and C_{PAH} in response to indomethacin emphasize the important role of renal prostaglandins in the control of renal hemodynamic function during sodium depletion. *Circ Res* 47: 99-107, 1980.

THE CONTROL of renin secretion is regulated by two intrarenal receptors, the renal sympathetic nerves and several humoral agents (Davis and Freeman, 1976). An acute decrease in renal perfusion pressure activates both the renal vascular receptor (so-called baroreceptor) and the macula densa, and in the denervated, nonfiltering kidney model in adrenalectomized dogs, the vascular receptor in the renal afferent arteriole appears to function autonomously (Blaine and Davis, 1971). It was reported by Larsson et al. (1974) for the rabbit and, more recently, by Data et al. (1978) for the dog that indomethacin blocked the renin response to renal artery or suprarenal aortic constriction. These findings and the new evidence (Seymour et al., 1979; Gerber et al., 1979) that renal arterial infusion of PGI_2 , PGD_2 , and PGE_2 increased renin release in the denervated nonfiltering dog kidney suggest that one or more of these prostaglandins act on either or both the renal juxtaglomerular (JG) cells and the renal vascular receptor. It is of interest that Terragno et al. (1978) have reported that PGI_2 is synthesized in the renal afferent arterioles and the interlobular arteries.

Recently, it was suggested (Oates et al., 1979)

that renin secretion is controlled by two major pathways which are mediated by (1) the adrenergic nervous system and (2) the renal prostaglandins. Our experiments were designed to examine the role of renal prostaglandins in the control of renin release in sodium-depleted dogs. The adrenergic nervous system in the kidney was rendered non-functional by bilateral renal denervation and subsequent propranolol administration, and the acute response in plasma renin activity (PRA) to indomethacin and meclofenamate was studied in conscious sodium-depleted dogs. Arterial pressure, renal hemodynamic function, and electrolyte metabolism were also measured; the results from these observations were helpful in defining the action of indomethacin on renin release. A group of conscious, normal, sodium repleted dogs was studied similarly as a control series. Finally, after administration of SQ 14,225 to increase PRA to a very high level in another group of conscious, sodium-depleted dogs with blockade of the adrenergic nervous system, indomethacin was given to enable us to study PRA and kidney function.

Methods

Female mongrel dogs (18-25 kg) were anesthetized with sodium pentobarbital (30 mg/kg, iv), and catheters were inserted into the femoral vessels and exteriorized through an incision at the back of the neck. For denervation, each kidney was isolated

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retroperitoneally via a flank incision, and the renal nerves were removed surgically from the renal vessels which were painted with a 5% phenol solution. In previous studies (Gotshall et al., 1973), this procedure reduced kidney norepinephrine content to near zero. In the present study, tissue norepinephrine content of the denervated kidneys was <0.05 $\mu\text{g/g}$ tissue compared to 0.20 ± 0.2 $\mu\text{g/g}$ tissue norepinephrine for normal control kidneys; the samples were analyzed by means of high pressure liquid chromatography (Davis et al., 1978). A perineotomy also was performed to facilitate bladder catheterization. The dogs were allowed to recover from surgery for at least 1 week, during which they were trained to lie quietly on a concave padded table. All were fed a diet containing 60 mEq sodium, and the urinary excretion of sodium was determined daily. Blood samples were collected in chilled tubes containing 0.1 ml 10% EDTA for determination of PRA. Separate blood samples were collected in sodium heparin (10 μm) for determination of plasma electrolytes.

Sodium depletion was accomplished by im injections of Mercurhydrin (4 mg mercury/kg as mercuriluride sodium and theophylline, 4.8 mg/kg on each of the first 2 days of a low salt diet (<3 mEq/day) which regimen was maintained throughout the study. This procedure resulted in an average negative sodium balance of 140 ± 4 mEq for each dog. Additional diuretic was administered periodically to ensure a marked degree of sodium depletion.

Five series of experiments were performed in the present study, during which the effects of prostaglandin synthetase inhibitors on PRA, mean arterial pressure, heart rate, renal function, and sodium and water excretion were studied in the conscious dog. For all experiments, effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) were measured by the respective clearances of *p*-aminohippurate (PAH) and creatinine (Cr). Some dogs were used in two or more experiments so the *n* values which follow apply to the number of experiments performed.

In group I ($n = 5$), normal, sodium-repleted dogs were studied as a control series. After two 45-minute control renal clearance periods, indomethacin (bolus of 5 mg/kg) was given intravenously, and observations were made for four additional 45-minute clearance periods. The indomethacin was prepared by dissolving the drug in 5 ml of ethyl alcohol and by adding 20 ml of phosphate buffer; the solution was adjusted to pH 8.3. Group II was studied in the same manner as group I, except that sodium-depleted animals with renal denervation were used ($n = 8$). The dogs studied in group II were also used in groups III and IV. In group III, all sodium-depleted dogs ($n = 6$) were given propranolol hydrochloride orally (25 mg/kg per day) in divided doses 3 times daily for at least 7 days to ensure complete blockade of β receptor-mediated renin release (Hanson et al., 1976); the experimental design be-

fore the acute experiment was the same as in group II except for the propranolol treatment. Also, the design of the acute experiment was the same as in group II, except that a supplemental dose of indomethacin (5 mg/kg bolus) was given 90 minutes after the first dose, and studies made for four additional 45-minute clearance periods. In group IV, the experimental design was the same as in group II, except that a bolus of meclofenamate of 5 mg/kg (prepared in the same way as the indomethacin) was given to inhibit prostaglandin synthesis. The fifth acute study (group V) was performed in five additional dogs subjected to bilateral renal denervation, sodium depletion, and propranolol administration. The experimental design was the same as in group IV, except that after the two control periods the angiotensin I-converting enzyme inhibitor (CEI) SQ 14,225 was given (10 mg/kg iv bolus followed by 10 $\mu\text{g/kg}$ per min constant infusion); observations then were made for two 45-minute renal clearance periods. After these observations, indomethacin was given in two doses (5 mg/kg each) 90 minutes apart; the first dose was followed by two periods, and the second dose was followed by four periods as in group III.

Blood pressure and heart rate were measured with a Statham P23Db pressure transducer and recorded on a Hewlett-Packard 7702B recorder. Plasma and urine electrolytes, Cr, and PAH were determined by standard analytical procedures. The serum propranolol concentration was assayed by Ayerst Laboratories in blood samples collected every 2 hours from 8 a.m. to 4 p.m. The radioimmunoassay technique of Sealey et al. (1974) was used to measure PRA. Statistical differences within groups were evaluated by analysis of variance, and significance was tested with Duncan's multiple range test.

Results

Effects of Chronic Sodium Depletion in Dogs with Renal Denervation before and during Chronic Propranolol Administration

The results revealed that sodium depletion in dogs with renal denervation resulted in increased PRA, heart rate, and plasma potassium concentration, whereas arterial pressure and the plasma sodium level were unchanged (Table 1). Superimposed chronic propranolol treatment did not influence significantly the plasma potassium level, arterial pressure, or PRA but did lower heart rate to within the normal range. The serum propranolol concentrations in blood collected at 8 a.m., 10 a.m., noon, 2 p.m., and 4 p.m. are presented in Table 2; the lowest average level of serum propranolol concentration recorded was 156 ng/ml.

Control Series, Group I

In an acute experiment on sodium-repleted, conscious dogs, indomethacin produced no discernible

TABLE 1 Sequential Changes in Blood Pressure, Heart Rate, PRA, and Plasma Electrolytes in Conscious, Sodium-Depleted Dogs with Bilateral Renal Denervation Prepared for Study of the Acute Response to Indomethacin ($n = 7$)

		MAP (mm Hg)	HR (beats/min)	PRA (ng of AI/ml per hr)	P _{Na} (mEq/liter)	P _K (mEq/liter)
Normal except for renal denervation		93 ±2	87 ±5	0.82 ±0.15	145.9 ±0.5	3.75 ±0.4
After subsequent sodium depletion		96 ±2	104 ±6*	7.84 ±0.89*	142.1 ±0.5	4.49† ±0.10
After subsequent treatment with propranolol (25 mg/kg per day orally)						
Days of propranolol administration	3	90 ±2	76 ±6‡	5.38 ±1.35†	143.9 ±0.7	4.25 ±0.13*
	5	90 ±2	82 ±5‡	6.28 ±1.06*	141.0 ±1.1	4.72 ±0.17*
	7	88 ±2	82 ±4‡	6.96 ±1.14*	142.6 ±0.8	4.78 ±0.13*

MAP = mean arterial pressure; HR = heart rate; PRA = plasma renin activity; P_{Na} = plasma sodium concentration; P_K = plasma potassium concentration. In this and subsequent tables, the values represent means ± SEM.

* $P < 0.01$ from normal, except for renal denervation.

† $P < 0.05$ from normal, except for renal denervation.

‡ $P < 0.05$ from sodium-depleted, denervated dogs.

effects on PRA, arterial pressure, heart rate, and renal hemodynamic function, or on sodium, potassium, and water excretion (Table 3). Plasma sodium concentration remained constant, whereas plasma potassium concentration fell significantly after indomethacin administration.

Acute Response to Indomethacin in Dogs with Renal Denervation and Sodium Depletion, Group II

These dogs were given indomethacin but were not on propranolol (Table 4). PRA appeared to fall, but the change was not significant. Cr clearance (C_{Cr}), PAH clearance (C_{PAH}), urine flow, and potassium excretion were reduced consistently, whereas arterial pressure and heart rate fell for only 1 period after indomethacin administration. Renal vascular resistance also increased significantly during only the first period after indomethacin injection. No significant changes were observed in sodium excretion or in plasma electrolyte concentrations.

TABLE 2 Serum Propranolol Concentrations (ng/ml) in Conscious, Sodium-Depleted Dogs with Renal Denervation ($n = 7$)

8 a.m.*	10 a.m.	12 Noon	2 p.m.	4 p.m.
365 ±62	665 ±160	998 ±255	547 ±116	156 ±57

↑

Drug given was 25 mg/kg per day 3 times a day at 8 a.m., 4 p.m., and 10 p.m.

* 8 a.m. concentration analyzed on blood collected prior to morning dose of drug.

Acute Response to Indomethacin in Dogs with Renal Denervation and Sodium Depletion during Chronic Propranolol Administration, Group III

The initial 5-mg bolus injection of indomethacin decreased PRA from an average value of 5.27 to 2.91 ng of A I/ml per hour after 90 minutes (Table 5); the supplemental dose of indomethacin reduced PRA further to 2.14 ng of A I/ml per hour, a 59% fall from control. This minimal value of 2.14 ng of A I/ml per hour is 3 times greater than the average value of 0.70 ng of A I/ml per hour in the normal conscious dogs (Table 3). Subsequently, PRA increased progressively and returned to near the average control level. Arterial pressure fell from an average control value of 86 mm Hg to as low as 69 mm Hg during the second period after the second dose of indomethacin and gradually returned to 82 mm Hg, which is near the control level; heart rate was unchanged. The clearances of Cr and PAH fell markedly from average control values of 53.4 and 175 ml/min, respectively, to as low as 19.1 and 78 ml/min, respectively, during the first period after the second injection of indomethacin; during the last three periods, both functions increased toward the control levels. Filtration fraction fell from 0.31 to 0.22 at the peak of the response. In spite of the low control rates of sodium and water excretion (no sodium or water loading was given), both urine flow and renal sodium excretion fell from 0.57 to 0.17 ml/min and from 2.96 to 0.84 μ Eq/min, respectively, at the peak response. Renal potassium excretion fell from an average control value of 63 to 5.3 μ Eq/min and returned to a level of 39 μ Eq/min by the last experimental period. Plasma sodium and potassium concentrations were unchanged after administration of indomethacin.

TABLE 3 Effects of Indomethacin Administration in Normal, Sodium-Repleted Conscious Dogs ($n = 5$)

	C _{Cr} (ml/min)	C _{PAH} (ml/min)	FF	U _V (ml/min)	E _{Na} (μEq/min)	E _K (μEq/min)	MAP (mm Hg)	HR (beats/min)	PRA (ng/ml per hr)	P _{Na} (mEq/liter)	P _K (mEq/liter)
C ₁	87.7	275.0	0.33	0.43	32.0	43.9	104	106	0.54	142.8	4.29
	±1.7	±14.8	±0.02	±0.06	±12.3	±10.1	±2	±6	±0.16	±0.8	±0.05
C ₂	91.5	275.2	0.31	0.39	41.6	35.2	102	91	0.86	143.3	4.17
	±5.0	±13.2	±0.02	±0.06	±12.2	±10.7	±2	±5	±0.18	±0.8	±0.05
<i>Indomethacin (5 mg/kg, iv bolus)</i>											
E ₁	83.0	232.6	0.37	0.41	55.1	24.8	107	88	0.83	142.7	3.91
	±9.7	±37.1	±0.03	±0.09	±20.2	±9.0	±2	±3	±0.21	±0.9	±0.05*
E ₂	95.1	254.9	0.35	0.36	41.1	24.6	105	84	1.03	144.0	3.76
	±8.0	±25.5	±0.02	±0.20	±23.1	±13.1	±1	±4	±0.27	±1.0	±0.08†
E ₃	89.5	253.9	0.35	0.52	60.3	30.9	104	81	1.08	143.9	3.84
	±7.2	±25.3	±0.03	±0.19	±28.6	±18.0	±2	±5	±0.34	±0.6	±0.05*
E ₄	93.0	291.8	0.35	0.45	46.1	37.6	109	82	0.88	144.7	3.87
	±11.1	±22.8	±0.03	±0.10	±12.5	±15.8	±2	±6	±0.27	±0.7	±0.06*

FF = filtration fraction; U_V = urine volume; E_{Na} = excreted sodium; E_K = excreted potassium. C₁ and C₂ and E₁, E₂, E₃, and E₄ represent successive control and experimental (after indomethacin) periods, respectively.

* $P < 0.05$; † $P < 0.01$.

Acute Response to Meclofenamate in Dogs with Renal Denervation and Sodium Depletion during Chronic Propranolol Administration, Group IV

The responses to meclofenamate appeared to be qualitatively and quantitatively similar to those observed after indomethacin administration with two exceptions; PRA fell more with the indomethacin and the filtration fraction failed to decrease with meclofenamate (Table 6). PRA fell from an average control value of 6.4 to 3.2 ng of A I/ml per hour during the second period after meclofenamate administration, and PRA returned to the control level during the last two experimental periods. Arterial pressure declined, but the change was significant only during the second experimental period; heart rate was unaltered. The clearances of Cr and PAH were significantly reduced after meclofenamate during three of the four periods for Cr and during all four periods for PAH. Urine flow and

potassium excretion both were reduced substantially during the first three periods after meclofenamate administration, but the low control level of sodium excretion was unchanged. Both plasma sodium and potassium concentrations were unchanged.

Acute Response to Indomethacin during Acute SQ 14,225 and Chronic Propranolol Administration in Dogs with Renal Denervation and Sodium Depletion, Group V

Angiotensin I-converting enzyme inhibition with SQ 14,225 produced a striking increase in PRA from an average value of 5.7 to 29.3 ng of A I/ml per hour (Table 7). Superimposition of indomethacin on converting enzyme inhibition appeared to lower PRA to 12.7 ng of A I/ml per hour after 90 minutes, and the supplemental dose of indomethacin failed to reduce PRA further; however, this change is not quite significant. It is interesting to note that this

TABLE 4 Effects of Indomethacin in Conscious, Sodium-Depleted Dogs with Bilateral Renal Denervation ($n = 8$)

	C _{Cr} (ml/min)	C _{PAH} (ml/min)	FF	U _V (ml/min)	E _{Na} (μEq/min)	E _K (μEq/min)	MAP (mm Hg)	RVR [mm Hg (ml/min) ⁻¹]	HR (beats/min)	PRA (ng/ml per hr)	P _{Na} (mEq/liter)	P _K (mEq/liter)
C ₁	66.9	216.6	0.30	0.60	2.89	57.8	95	0.31	105	6.15	142.2	4.86
	±7.3	±21.4	±0.2	±0.07	±1.11	±4.0	±3	±0.04	±6	±.93	±0.7	±0.15
C ₂	64.5	214.5	0.29	0.61	3.95	59.3	92	0.32	99	5.36	141.7	4.71
	±4.9	±24.0	±0.02	±0.08	±1.73	±5.5	±3	±0.04	±9	±0.92	±0.7	±0.12
<i>Indomethacin (5 mg/kg, iv bolus)</i>												
E ₁	36.8	101.8	0.33	0.25	1.33	15.3*	92	0.60	101	4.59	143.1	4.48
	±3.5*	±10.1*	±0.01	±0.01*	±0.39†	±2.1	±4	±0.07†	±9	±1.41	±0.7	±0.08
E ₂	26.0	90.7	0.26	0.19	0.83	4.5	86	0.94	93	4.31	143.0	4.53
	±3.0*	±11.5*	±0.01	±0.01*	0.31†	±1.1*	±4	±0.41	±8	±0.99	±0.7	±0.10
E ₃	30.9	138.6	0.25	0.26	1.22	10.7	80	0.57	91	4.46	143.4	4.65
	±4.3*	±9.6*	±0.02	±0.02*	±0.30†	±3.5*	±2*	±0.19	±8†	±1.07	±1.0	±0.08
E ₄	45.4	165.1	0.27	0.37	1.58	29.3	86	0.37	93	5.67	143.7	4.68
	±4.3*	±15.4	±0.02	±0.03*	±0.37†	±7.1*	±3	±0.05	±10	±1.05	±1.0	±0.14

RVR = renal vascular resistance. C₁, C₂, E₁-E₄ as defined in Table 3.

* $P < 0.01$; † $P < 0.05$.

TABLE 5 Effects of Indomethacin with Supplemental Administration Treatment of the Inhibitor in Conscious, Sodium-Depleted Dogs with Bilateral Renal Denervation during Chronic Propranolol ($n = 7$)

	C _{Cr} (ml/ min)	C _{PAH} (ml/ min)	FF	U _V (ml/ min)	E _{Na} (μEq/ min)	E _K (μEq/ min)	MAP (mm Hg)	RVR [mm Hg (ml/ min) ⁻¹]	HR (beats/ min)	PRA (ng/ml per hr)	P _{Na} (mEq/ liter)	P _K (mEq/ liter)
C ₁	53.1 ±6.6	175.0 ±17.0	0.30 ±0.02	0.58 ±0.06	2.73 ±0.72	68.9 ±14.1	87 ±3	0.33 ±0.02	79 ±3	4.77 ±0.68	142.4 ±0.7	4.65 ±0.25
C ₂	53.7 ±4.9	176.1 ±8.4	0.31 ±0.02	0.55 ±0.07	3.20 ±1.14	57.1 ±4.5	85 ±2	0.33 ±0.01	85 ±4	5.71 ±1.14	143.1 ±0.5	4.84 ±0.16
<i>Indomethacin (5 mg/kg, iv bolus)</i>												
E ₁	28.8 ±3.3*	92.4 ±9.0*	0.31 ±0.02	0.21 ±0.02*	1.38 ±0.44†	12.6 ±2.5*	75 ±5†	0.61 ±0.07†	81 ±3	3.17 ±0.53	144.2 ±0.6	4.59 ±0.15
E ₂	24.6 ±3.3*	102.7 ±9.3*	0.24 ±0.02†	0.21 ±0.02*	0.84 ±0.22†	7.7 ±2.0*	72 ±4*	0.47 ±0.04†	79 ±4	2.91 ±0.56†	144.5 ±0.6	4.80 ±0.14
<i>Indomethacin (5 mg/kg, iv bolus)</i>												
E ₃	19.1 ±3.0*	78.0 ±10.5*	0.26 ±0.02†	0.17 ±0.02*	0.85 ±0.25†	5.3 ±0.8*	69 ±5*	0.68 ±0.13†	79 ±5	2.14 ±0.39†	144.6 ±0.8	4.77 ±0.18
E ₄	22.2 ±3.5*	97.2 ±9.7*	0.22 ±0.02*	0.25 ±0.04*	0.98 ±0.12†	12.2 ±5.5*	69 ±4*	0.49 ±0.03*	79 ±6	2.70 ±0.80†	145.1 ±1.0	4.84 ±0.19
E ₅	29.0 ±5.8*	116.9 ±10.4*	0.24 ±0.03†	0.31 ±0.03*	1.26 ±0.23†	19.1 ±8.5*	75 ±4†	0.42 ±0.07	80 ±6	3.91 ±1.25	145.2 ±0.90	4.91 ±0.18
E ₆	40.0 ±3.3†	141.8 ±6.0†	0.28 ±0.02	0.46 ±0.04†	2.62 ±0.63	39.3 ±7.9†	82 ±3	0.38 ±0.01	80 ±8	5.05 ±1.38	145.3 ±0.8	4.86 ±0.25

C₁ and C₂ and E₁, E₂, E₃, E₄, E₅ and E₆ represent successive control and experimental (after indomethacin) periods, respectively.* $P < .01$ from control; † $P < 0.05$ from control.

apparent 58% fall in PRA from the values recorded during the initial SQ 14,225 administration is almost identical to the percentage reduction that occurred in the absence of converting enzyme inhibition (Table 5). Arterial pressure fell from 98 mm Hg to an average of 72 mm Hg after SQ 14,255 administration alone. There appeared to be a further fall in arterial pressure after the second dose of indomethacin, but the declines to 64 and 60 mm Hg were not significant. Heart rate was unchanged throughout the experiment. Renal vascular resistance decreased in response to SQ 14,225 and then increased compared with the first two periods after SQ 14,225 administration.

The clearance of PAH was not changed significantly during converting enzyme inhibition alone, but the C_{Cr} fell during the first period after SQ 14,225 injection. After indomethacin, C_{Cr} was reduced during four of the six periods, and C_{PAH} was decreased in five of the six periods compared with the data obtained after SQ 14,225 but before indomethacin injection. Filtration fraction was reduced from 0.29 to 0.15 by converting enzyme inhibition alone; this was followed after indomethacin injection by an apparent increase in filtration fraction to as high as 0.26 and, subsequently, a fall to the low level of 0.16. Urine flow, sodium and potassium excretion, and plasma sodium and potassium con-

TABLE 6 Effects of Meclofenamate in Conscious, Sodium-Depleted Dogs with Renal Denervation on Chronic Propranolol Treatment ($n = 6$)

	C _{Cr} (ml/ min)	C _{PAH} (ml/ min)	FF	U _V (ml/ min)	E _{Na} (μEq/ min)	E _K (μEq/ min)	MAP (mm Hg)	RVR [mm Hg (ml/ min) ⁻¹]	HR (beats/ min)	PRA (ng/ml per hr)	P _{Na} (mEq/ liter)	P _K (mEq/ liter)
C ₁	58.1 ±6.7	192.4 ±17.3	0.30 ±0.02	0.64 ±0.08	3.68 ±1.6	71.4 ±14.0	86 ±2	0.29 ±0.04	81 ±5	5.7 ±1.5	142.5 ±0.9	4.92 ±0.12
C ₂	56.4 ±6.2	210.8 ±20.6	0.27 ±0.01	0.66 ±0.12	3.08 ±1.7	6.39 ±16.0	84 ±3	0.29 ±0.03	87 ±5	7.1 ±2.2	142.4 ±0.9	4.71 ±0.18
<i>Meclofenamate (5 mg/kg, iv bolus)</i>												
E ₁	27.4 ±7.4*	88.7 ±17.4*	0.30 ±0.02	0.21 ±0.03*	2.23 ±1.7	12.9 ±2.8†	77 ±5	0.68 ±0.10†	84 ±4	4.2 ±0.9	144.5 ±1.2	4.51 ±0.15
E ₂	24.6 ±7.3*	97.0 ±15.7*	0.24 ±0.03	0.20 ±0.01*	0.71 ±0.22	5.6 ±1.7*	71 ±6†	0.58 ±0.07*	85 ±7	3.2 ±0.8†	144.0 ±1.0	4.66 ±0.14
E ₃	41.0 ±6.8†	162.6 ±16.3†	0.25 ±0.02	0.39 ±0.04†	1.28 ±0.44	27.4 ±7.0†	78 ±5	0.33 ±0.03	82 ±7	6.6 ±1.7	144.0 ±1.1	4.54 ±0.17
E ₄	51.5 ±8.3	175.0 ±20.9	0.29 ±0.02	0.65 ±0.11	2.07 ±0.44	58.1 ±15.7	85 ±5	0.33 ±0.03	85 ±5	6.8 ±1.9	143.6 ±0.8	4.45 ±0.18

C₁ and C₂ and E₁, E₂, E₃, and E₄ represent successive control and experimental periods (after meclofenamate), respectively.* $P < 0.01$; † $P < 0.05$.

TABLE 7 Acute Effects of SQ 14,225 and Indomethacin in Conscious, Sodium-Depleted Dogs on Chronic Propranolol Treatment ($n = 5$)

	C _{Cr} (ml/ min)	C _{PAH} (ml/ min)	FF	U _V (ml/ min)	E _{Na} (μEq/ min)	E _K (μEq/ min)	MAP (mm Hg)	HR (beats/ min)	PRA (ng/ml per hr)	P _{Na} (mEq/ liter)	P _K (mEq/ liter)	RVR [mm Hg (ml/ min) ⁻¹]
C ₁	67.0	233.2	0.30	0.66	1.56	37.2	97	81	5.3	139.7	4.92	0.28
	±9.5	±33.3	±0.02	±0.14	±0.42	±9.7	±3	±4	±1.6	±0.9	±0.19	±0.5
C ₂	66.7	249.5	0.28	0.56	1.28	30.6	98	85	6.1	139.6	4.75	0.26
	±10.3	±41.6	±0.04	±0.13	±0.40	±7.8	±2	±3	±2.3	±0.9	±0.21	±0.05
<i>SQ 14,225 (10 mg/kg, iv bolus and 10 μg/kg per min)</i>												
C ₃	37.5	235.9	0.16	0.33	1.78	24.2	70	84	29.6	141.0	4.66	0.20
	±7.2*	±24.1	±0.3	±0.10	±0.38	±9.8	±2†	±2	±8.3†	±1.0	±0.20	±0.02
C ₄	48.8	339.7	0.15	0.37	1.83	26.5	74	85	29.0	140.6	4.67	0.14
	±6.4	±34.0	±0.02*	±0.04	±0.31	±7.0	±2†	±1	±7.6†	±0.8	±0.19	±0.02*
<i>Indomethacin (5 mg/kg, iv bolus)</i>												
E ₁	34.8	158.0	0.25	0.27	2.14	16.5	70	79	16.4	141.2	4.58	0.32
	±5.1*	±34.0	±0.04	±0.02	±0.80	±2.4	±2†	±3	±3.9	±0.9	±0.20	±0.07‡
E ₂	44.2	221.9	0.22	0.32	1.39	19.8	70	81	12.7	141.6	4.56	0.20
	±4.8	±38.4‡	±0.03	±0.03	±0.30	±7.2	±2†	±3	±2.8	±1.0	±0.24	±0.03
<i>Indomethacin (5 mg/kg, iv bolus)</i>												
E ₃	43.7	183.2	0.26	0.32	1.54	20.6	70	78	12.2	142.4	4.58	0.26
	±7.3	±30.7‡	±0.04	±0.02	±0.34	±6.7	±3†	±3	±3.8	±1.0	±0.26	±0.05‡
E ₄	35.1	148.3‡	0.20	0.29	1.39	18.1	65	78	12.1	143.0	4.59	0.22
	±4.4*	±8.9	±0.02*	±0.02	±0.30	±6.9	±3†	±3	±3.8	±1.0	±0.24	±0.02‡
E ₅	28.1	190.0	0.16	0.29	1.10	13.6	64	78	14.5	142.7	4.57	0.22
	±2.3*	±24.0‡	±0.02*	±0.01	±0.22	±3.6	±2†	±6	±4.9	±1.3	±0.26	±0.03‡
E ₆	35.6	208.6	0.18	0.35	1.22	16.4	60	76	16.1	142.1	4.67	0.19
	±5.7*	±33.7‡	±0.02*	±0.02	±0.24	±4.7	±3†	±5	±4.9	±1.0	±0.29	±0.02

C₁ and C₂ represent control periods before SQ 14,225 and C₃ and C₄ control periods before indomethacin; periods E₁–E₆ are after indomethacin.

* $P < 0.05$ from C₁ + C₂; † $P < 0.01$ from C₁ + C₂; ‡ $P < 0.05$ from C₄.

centrations were not significantly changed during converting enzyme inhibition alone or during superimposed indomethacin administration.

Discussion

The importance of renal prostaglandins in the control of renin release has been studied extensively during the last 5 years, and arguments have been presented for two major control mechanisms, the adrenergic nervous system and the renal prostaglandins (Oates et al., 1979). This group reported that hypertensive patients on a 10 mEq/day sodium diet who were given propranolol responded to indomethacin with a striking decrease in PRA. In normal subjects, studied similarly on a low sodium diet, but without propranolol treatment, indomethacin failed to reduce PRA. Their observations (Gerber et al., 1978) that PGI₂ infused into the renal artery of dogs increased renin release, whereas PGD₂ (when studied similarly) failed to produce a response with the same degree of vasodilation, along with several other findings, led Oates and co-workers (1979) to suggest that it is PGI₂ that controls renin secretion.

Our study was designed to examine the effects of indomethacin in conscious sodium-depleted dogs with blockade of the adrenergic nervous system. Terragno and associates (1977) have emphasized the importance of studying renal prostaglandins in

conscious dogs because laparotomy and anesthesia increased renal prostaglandin release in this species. In the present sodium-depleted dogs with adrenergic blockade but before indomethacin, PRA was elevated 7- to 9-fold above the average normal level. It is clear, therefore, as reported previously by Gotshall et al. (1973) for sodium depletion, that during blockade of the adrenergic nervous system there are major control mechanisms present for increasing renin release. Also, there is evidence (Davila et al., 1978) that renal prostaglandin release is increased in the sodium-depleted state in the rabbit.

In the present group II dogs with renal denervation but that did not receive propranolol, PRA appeared to decline slightly after indomethacin administration, but the change was not significant. This failure of PRA to decrease might reflect the presence and action of increased plasma norepinephrine on the JG cells since plasma norepinephrine is increased during sodium depletion (Robertson et al., 1977). When virtually all adrenergic input into the JG cells and renal vascular receptor was blocked by adding propranolol to renal denervation (group III), a striking 59% decrease in PRA occurred after indomethacin administration. It should be emphasized, however, that PRA was still 3 times the normal level (see Table 3).

The doses of indomethacin used in this study are

large, and it seems likely that prostaglandin synthetase inhibition was virtually complete after the second dose. It has been demonstrated that PGE_2 in renal venous blood (Terragno et al., 1977; Zambraski and Dunn, 1979) and urinary PGE_2 excretion (Zambraski and Dunn, 1979) in the dog are markedly reduced with doses of indomethacin much smaller than those used here. More recently, Fröllich et al. (1979) have reported a marked decrease in the urinary excretion of a major metabolite of PGE_1 and PGE_2 in response to indomethacin in sodium-depleted, hypertensive patients receiving propranolol. This situation parallels very closely the present conditions in sodium-depleted dogs with blockade of the adrenergic nervous system, and the responses in PRA to indomethacin in man and dog are quite similar.

Since arterial pressure fell from a control level of 86 mm Hg to 69 mm Hg after the second dose of indomethacin in group III, the renal vascular receptor might have helped to sustain the 3-fold elevation in PRA. If this interpretation is correct, then the renal vascular receptor does not appear to be fully dependent on renal prostaglandins to carry out its function. Data et al. (1978) have provided evidence that indomethacin blocked the renin response to aortic constriction in dogs with a denervated, non-filtering kidney, but the level of renal perfusion pressure (57–61 mm Hg) was below the autoregulatory range. In two recent studies (Seymour and Zehr, 1979; Blackshear et al., 1979), both groups reported that intrarenal indomethacin administration failed to attenuate the renin response to aortic constriction when pressure was reduced below the autoregulatory range. In addition, Blackshear et al. (1979) found that prostaglandin synthetase inhibition within the autoregulatory range for arterial pressure (120–90 mm Hg) attenuated the renin response to aortic constriction. All three studies were done in anesthetized animals and it would be interesting to see the results of similar studies in conscious dogs. Also, in the present study, it seems likely that the macula densa was activated and helped to sustain the elevated level of PRA during inhibition of prostaglandin synthesis since both the filtered load of sodium and renal sodium excretion were markedly reduced.

To examine further the role of renal prostaglandins in the control of renin release in sodium-depleted dogs with adrenergic blockade, angiotensin I converting enzyme was inhibited with SQ 14,225 before and during indomethacin administration; PRA increased from the high level of 5.7 to the extremely high level of 29.3 ng of A I/ml per hour during angiotensin blockade. At this level of PRA, the two 5-mg bolus injections of indomethacin appeared to reduce PRA by 58%, but the important result is that the absolute level of PRA was 12.1, which is 17 times the normal level. These observations suggest that other important mechanisms than the adrenergic nervous system and renal pros-

taglandins are operative to maintain the high level of 12.1. Again, both the renal vascular receptor and the macula densa must be considered as possible mechanisms which sustained the high level of PRA. The observations that the fall in PRA was 58–59%, regardless of the initial level of PRA of 5.24 ng of A I/ml per hour (Table 5) and 29.3 ng of A I/ml per hour (Table 7), suggest that prostaglandins modulate the control of renin release and that their role is permissive rather than initiative. McGiff et al. (1978) have repeatedly suggested a modulatory role for prostaglandins in the regulation of kidney function. Finally, it should be pointed out that SQ 14,225 is an inhibitor of kininase II, but evidence is lacking to demonstrate an effect to increase plasma kinins under these circumstances.

The experiment with meclofenamate demonstrated that the responses were qualitatively and quantitatively similar to those observed with indomethacin; this finding provides evidence for the specificity of the action of indomethacin on prostaglandin synthetase. Failure of a response to indomethacin to occur in the normal dogs (except for a decrease in plasma potassium concentration) also supports the idea of a specific action of indomethacin; others (Kirschenbaum and Stein, 1976) have previously reported that inhibitors of prostaglandin synthesis failed to influence renal hemodynamic function in normal conscious dogs, but they observed an increase in renal sodium excretion.

The striking decreases in C_{Cr} and C_{PAH} during indomethacin (group III) and meclofenamate (group IV) point to an important role for prostaglandins in the control of renal vascular resistance during sodium depletion. It seems likely that constriction of both the afferent and efferent renal arterioles occurred. In an earlier study from our laboratory (Seymour et al., 1979), infusions of PGI_2 and PGD_2 into the renal artery of dogs with a denervated, nonfiltering kidney increased renin release and increased renal blood flow. Consequently, in the present experiments, the action of indomethacin to result in constriction of the renal arterioles might reflect inhibition of synthesis of both PGI_2 and PGD_2 in the kidney. Since angiotensin II acts on the renal arterioles, a decrease in renal arteriolar prostaglandins would allow the angiotensin II present to increase renal vascular resistance. Failure of renal blood flow to decrease with indomethacin in normal dogs might be a reflection of the minor role of renal prostaglandins and of angiotensin II in the physiological control of blood flow in the kidney.

Recently, Gerber et al. (1979) have demonstrated an increase in renin release with PGI_2 and, in addition, with PGE_2 and 13,14-dihydro PGE_2 in dogs given propranolol and with a nonfiltering kidney. Since PGI_2 , PGE_2 , and PGD_2 are all synthesized from C^{14} arachidonic acid by renal cortical microsomes (Whorton et al., 1978), it is conceivable that all three compounds contributed to the release of

renin during sodium depletion. However, the fact that PGI_2 is synthesized by the afferent arteriole and is more potent in releasing renin than either PGD_2 (Seymour et al., 1979) or PGE_2 (Gerber et al., 1979) favors a primary role for PGI_2 .

There is the question of the site of action of the renal prostaglandins to increase renin release; do they act directly on the JG cells, on the vascular receptor in the renal afferent arteriole, or on both? Osborn et al. (1978) have suggested a direct action of PGE_2 on the JG cells since other vasodilators, such as acetylcholine, bradykinin, and eledoisin, increased renal blood flow as much as infusion of PGE_2 but did not increase renin release. Similar results were obtained by Gerber et al. (1979) with intrarenal papaverine while studying the vasodilator action of PGI_2 and PGE_2 . Furthermore, Whorton et al. (1977) demonstrated that PGI_2 increased renin release from renal cortical slices. Collectively, these observations point to an action at the JG cell level, but additional studies are needed to exclude an action of prostaglandins on the renal vascular receptor.

One of the most striking hemodynamic changes that occurred in response to indomethacin was the fall in arterial pressure. In conscious sodium-depleted dogs, it has recently been demonstrated (Stephens et al., 1978) that arterial pressure is sustained by increased total peripheral resistance in the presence of a fall in cardiac output. This occurs in association with an increase in PRA and the action of angiotensin II on the peripheral arterioles. In response to indomethacin, as PRA fell, arterial pressure also decreased; and, as the effect of indomethacin declined, both PRA and arterial pressure increased. A high correlation ($P < 0.005$) between PRA and mean arterial pressure was observed before and after indomethacin or meclofenamate administration (from the data in Tables 4-6). It appears, therefore, that arterial pressure fell as the support from the action of angiotensin II on the peripheral arterioles was decreased by the decline in PRA.

The present findings are consistent with the observations of Oates and associates (1979) that indomethacin decreased PRA in sodium-depleted hypertensive patients during propranolol administration. However, in the present study, when angiotensin II formation was blocked in denervated, propranolol-treated, sodium-depleted dogs, PRA never fell below 12 ng of A I/ml per hour in response to indomethacin. This level of PRA is 17 times normal and reflects the action of mechanisms other than the adrenergic nervous system and the renal prostaglandins. Also, these observations may indicate that the renal prostaglandins play a permissive role and modulate the control of renin release. In dogs with blockade of the adrenergic nervous system and indomethacin administration, both the renal vascular receptor mechanism and the macula densa might be operative to increase renin release. The

present studies also are consistent with the suggestion of several workers that PGI_2 is an important renal prostaglandin involved in renin release. The present striking decreases in C_{cr} , C_{PAH} , and filtration fraction in response to indomethacin emphasize the important role of renal prostaglandins in control of renal hemodynamic function during sodium depletion.

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