Role of Adrenoceptors in Diphenylhydantoin-Stimulated Renin Release (42870)

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Abstract. **We have previously shown that diphenylhydantoin (DPH)-stimulated renin release is mediated by, or requires the presence of, the renal nerves. In the present study, we examined the effects of adrenergic blockers in DPH-stimulated renin release in five groups of anesthetized dogs. In vehicle-treated dogs, DPH at a dose of 0.18 mg/kg-min increased renin secretion rate (RSR) from 56 ± 14 to 269 ± 60 and returned to 84 ± 30 ng of angiotensin (ANG) I/hr-min ($P < 0.01$, analysis of variance). In metoprolol-treated dogs, DPH produced no significant changes in RSR (90 ± 28 to 144 ± 67 to 100 ± 51 ng of ANG I/hr-min). Likewise, in atenolol-treated dogs, RSR was 34 ± 10 before, 59 ± 15 during, and 23 ± 8 ng of ANG I/hr-min after the infusion of DPH. In contrast, after pretreatment with ICI 118,551 (a β_2 adrenoceptor antagonist), RSR was 37 ± 9 before, 151 ± 57 during, and 47 ± 12 ng of ANG I/hr-min after the infusion of DPH ($P < 0.01$). In phentolamine-treated dogs, RSR was 69 ± 20 before, 295 ± 53 during, and 95 ± 17 ng of ANG I/hr-min after the infusion of DPH ($P < 0.01$). Changes in renal blood flow, renal vascular resistance, and $U_{Na}V$ were in the same directions in all groups. These data suggest that DPH-stimulated renin release is mediated by β_1 adrenoceptors since both β_2 and α adrenoceptor antagonists have no effects on DPH-stimulated renin release.** [P.S.E.B.M. 1989, Vol 190]

Diphenylhydantoin (DPH) has been shown to correct neural hyperexcitability and is used clinically for control of seizures. Previous studies indicate that DPH stimulates renin release from rat renal cortical slices (1) and in intact dogs (2, 3). This stimulatory effect of DPH on renin release in intact dogs was abolished by prior renal denervation whether the denervation was done acutely or 24 hr prior to the administration of DPH (2). This finding suggests that DPH-stimulated renin release is mediated by, or requires, the presence of the renal nerves.

Recent studies suggest that the renal sympathetic nerves play an important role in mediating renin release. Renal nerve stimulation increases renin release in the absence of changes in renal perfusion pressure, renal blood flow, glomerular filtration rate or urinary sodium excretion (4). This stimulatory effect is abolished by β_1 adrenoceptor blockade with atenolol (4) or with metoprolol (5) but is unaffected by β_2 adrenoceptor blockade with butoxamine (4) or ICI 118,551 (6). Similarly, renal α adrenoceptor blockade with phentolamine fails to affect renin release in response to renal nerve stimulation (7). Taken together, these results tend to suggest that renal nerve-stimulated renin release in the dog is mediated by β_1 adrenoceptors.

This study was designed to evaluate further the role of the renal nerves in DPH-stimulated renin release in the dog to determine whether this effect is mediated by α or β adrenoceptors.

Materials and Methods

The study was performed in five groups of adult, female mongrel dogs weighing 18–25 kg. Food was withheld for 18 hr prior to the study, but the animals were allowed free access to water. On the day of the study, the animals were anesthetized with intravenous sodium pentobarbital (30 mg/kg), intubated, ventilated with a positive pressure respirator (Harvard Apparatus, Milis, MA). Supplemental doses of sodium pentobarbital were administered intravenously as needed to maintain a stable state of anesthesia. Following anesthesia, a polyethylene catheter was placed in the left renal vein for blood sampling. An electromagnetic flow probe was placed around the left renal artery for con-

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tinuous monitoring of renal blood flow with an electromagnetic flow meter (Carolina Medical Electronics, King, NC). Two 23-gauge needles were inserted into the left renal artery for infusion of DPH or adrenergic agents and were maintained with a continuous infusion of 2.5% dextrose in water. A brachial artery catheter connected to a PD 230 Statham transducer (Statham Instruments, Oxnard, CA) was used for continuous monitoring of systemic blood pressure with a Gilson recorder. Following the induction of anesthesia, each animal received a maintenance intravenous infusion of 4 ml/min of 2.5% dextrose in water through a forearm vein. After surgery, an intravenous infusion of 0.9% saline (0.5 ml/min) containing sufficient inulin to maintain a blood level of 20–25 mg/dl was given. A 60-min stabilization period was allowed to establish a baseline.

After the stabilization period, three 5-min urine collections were made in all dogs (first control period). During these and all subsequent periods, arterial and renal venous bloods were collected during the middle of the first and third such periods of urine collection and analyzed for inulin, plasma renin activity, Na^+, and K^+. After these clearance periods, DPH (0.18 mg/kg-min) was added to one of the intrarenal arterial infusates and continued for 20 min in all groups. Another three clearance periods were established while the DPH infusion continued (first DPH period). The DPH infusion was then discontinued and 30 min later another three clearance periods were obtained (first recovery period) in all groups. All groups then received an intrarenal arterial infusion of 2.5% dextrose in water via this renal arterial line. Fifteen minutes later, three 5-min clearance periods were observed (second control period). Then DPH in the previous dose was added again for 20 min, after which three 5-min clearance periods were observed while the DPH continued (second DPH period). The DPH was stopped and the 2.5% dextrose in water was continued. After 30 min, the final three clearance periods were begun (second recovery period).

In all groups, the second intrarenal arterial infusate was 2.5% dextrose in water through the end of the first recovery period. The five groups differed only in what was infused via this line, after this point. Group 1 had the 2.5% dextrose in water continued to the end of the protocol. Groups 2 through 5 received adrenergic blockers dissolved in 2.5% dextrose in water via this route for the remainder of the protocol: metoprolol (2 μg/kg-min, Group 2) atenolol (2 μg/kg-min, Group 3), ICI 118,551 (2 μg/kg-min, Group 4), and phentolamine (8 μg/kg-min, Group 5). The doses of atenolol and metoprolol were the same as those used previously (4, 5). The dose of ICI 118,551 used inhibits isoproterenol (2 ng/kg)-stimulated renin release.

The analytical procedures and calculations used in the present experiments have been described previously (3). Renin secretion rate (RSR) was calculated from (renal venous plasma renin activity − arterial plasma renin activity) × renal plasma flow and is expressed as nanograms (ng) of angiotensin (ANG) I per hour per minute. Renal vascular resistance (RSR) was calculated from the ratio of systemic blood pressure/renal blood flow and expressed as mm Hg/ml-min. All data are expressed as mean ± SE.

For each protocol, data from the first and second DPH infusions were analyzed separately for overall statistical significance by one-way analysis of variance. In cases in which the F value was significant, further analysis between the control and the DPH infusion periods was carried out using Scheffe's test (8).

Results

The results of this study are shown in Figure 1 and Table I. In Group 1, the first DPH infusion was associated with a reversible rise in RSR (80 ± 36 to 324 ± 78 to 63 ± 16 ng of ANG I/hr-min, $P < 0.01$, analysis of variance and <0.01, Scheffe's test, respectively). Similar results were seen with the second DPH infusion (56 ± 14 to 269 ± 60 to 84 ± 30 ng of ANG I/hr-min, $P <$ 0.01 and <0.01, respectively). Systemic blood pressure, renal blood flow, renal vascular resistance, and glomerular filtration rate remained fairly stable during both DPH infusions. Both infusions were accompanied by a rise in $U_{Na}V$.

In Group 2, first DPH infusion resulted in a reversible rise in RSR (80 ± 20 to 316 ± 95 to 127 ± 40 ng of ANG I/hr-min, $P < 0.05$ and <0.01, respectively). But when the dogs were pretreated with metoprolol,

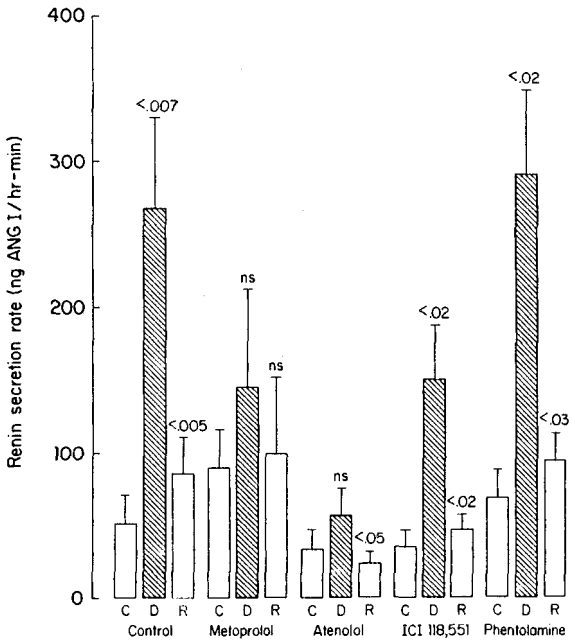

Figure 1. Renin secretion rates in response to diphenylhydantoin in control, metoprolol-, atenolol-, ICI 118,551-, and phentolamine-treated dogs. C, control; D, diphenylhydantoin infusion; R, recovery. * $P < 0.01$ compared with the preceding control period.

Table I. Effects of DPH-Adrenergic Blockers on Renal Hemodynamics and Electrolyte Excretion

	Systemic pressure (mm Hg)					Renal blood flow (ml/min)					RVR mm Hg (ml/min)$^{-1}$					Cin (ml/min)					$U_{Na}V$ (μEq/min)					U_KV (μEq/min)				
	C	D	R	C	D	R	C	D	R	C	D	R	C	D	R	C	D	R	C	D	R	C	D	R	C	D	R	C	D	R
Group 1: diphenylhydantoin (D) and D alone ($n = 6$)																														
Mean	142	140	147	146	141	142	213	230	213	212	233	208	0.68	0.62	0.74	0.73	0.64	0.71	24.3	27.3	28.4	28.1	28.3	29.0	12	45**	15	16	51**	32	17	25	28	29	35	36
±SE	7	7	6	6	5	4	13	13	20	20	18	16	0.06	0.05	0.10	0.10	0.08	0.08	2.4	1.8	1.7	1.7	2.3	1.9	4	13	4	5	13	7	2	3	4	4	5	5
Group 2: D and D + metoprolol ($n = 5$)																														
Mean	142	140	145	137	123*	125	223	236	220	215	216	217	0.66	0.62	0.69	0.66	0.58	0.59	28.5	30.6	30.3	31.2	29.4	29.1	30	60	24	29	55	23	18	24	25	28	28	29
±SE	7	6	5	2	3	4	20	23	25	20	12	19	0.07	0.06	0.06	0.05	0.03	0.05	2.6	2.9	3.2	2.2	1.7	1.6	17	17	10	13	15	8	3	3	5	7	8	6
Group 3: D and D + atenolol ($n = 6$)																														
Mean	128	121	132	133	121**	126	197	206	188	178	197	169	0.70	0.55	0.70	0.73	0.62	0.68	23.5	24.4	24.8	23.9	23.4	18.5	27	40	9	15	49**	26	17	19	19	23	28	32
±SE	4	3	4	3	2	3	27	17	19	21	29	13	0.12	0.05	0.07	0.09	0.08	0.08	3.5	3.4	2.9	2.8	2.7	3.7	12	19	3	7	10	10	3	2	2	3	4	4
Group 4: D and D + ICI 118, 551 ($n = 6$)																														
Mean	152	148	151	150	143	143	200	210	190	200	224	214	0.74	0.63	0.82	0.76	0.66	0.69	21.6	26.7	25.0	27.7	27.6	24.5	18	65	8	15	88**	31	16	22	19	26	36	38
±SE	5	5	6	5	4	5	13	17	18	21	24	26	0.05	0.06	0.07	0.07	0.07	0.08	4.3	2.2	2.6	2.4	2.6	2.4	6	27	3	6	32	12	3	4	4	4	6	5
Group 5: D and D + phentolamine ($n = 5$)																														
Mean	141	139	141	139	132	135	157	174	145	149	186	173	0.90	0.80	0.99	0.96	0.72	0.84	21.5	22.3	21.5	23.1	23.5	22.3	27	72	15	7	84**	17	11	16	15	18	23	23
±SE	12	11	9	7	7	7	9	9	13	14	13	24	0.07	0.05	0.07	0.07	0.03	0.11	0.5	1.6	2.1	1.5	1.6	0.5	21	35	9	2	28	2	2	3	1	3	4	4

Note. Values are mean ± SE. Cin, inulin clearance; $U_{Na}V$, U_KV, urinary excretion of sodium and K; C, control; D, DPH infusion; R, recovery. Adrenergic blockers were given throughout the second half of each experiment.
*<0.25, ** <0.01 significantly different from preceding period.

DPH produced no significant changes in RSR (90 ± 28 to 144 ± 67 to 100 ± 51 ng of ANG I/hr-min, $P > 0.05$ for each) despite a drop in systemic blood pressure. Neither infusion was associated with significant changes in renal blood flow, RVR, glomerular filtration rate, $U_{Na}V$, or U_KV.

In Group 3, DPH produced a reversible increase in RSR (152 ± 47 to 312 ± 84 to 70 ± 36 ng of ANG I/hr-min, $P < 0.05$ and <0.05, respectively) during the first infusion. During the second infusion when the animals were pretreated with atenolol, the RSR were 34 ± 10 before, 59 ± 15 during, and 23 ± 8 ng of ANG I/hr-min after DPH infusion, despite a fall in systemic blood pressure. Urinary sodium excretion increased significantly during the second DPH infusion.

In Group 4, the first DPH infusion was associated with a reversible rise in RSR (107 ± 58 to 253 ± 89 to 75 ± 26 ng of ANG I/hr-min). After pretreatment with ICI 118,551, DPH produced a similar effect (37 ± 9 to 151 ± 37 to 47 ± 12 ng of ANG I/hr-min, $P < 0.01$ and <0.01, respectively). Systemic blood pressure, renal blood flow, RVR, and U_KV did not change significantly with either infusion. $U_{Na}V$ increased significantly during the second infusion.

In Group 5, a reversible rise in RSR was seen with the first DPH infusion (75 ± 23 to 304 ± 71 to 94 ± 33 ng of ANG I/hr-min, $P < 0.01$ and <0.01, respectively). After treatment with phentolamine, DPH produced similar results (69 ± 20 to 295 ± 53 to 95 ± 17 ng of ANG I/hr-min, $P < 0.01$ and <0.01, respectively). Systemic blood pressure, renal blood flow, RVR, glomerular filtration rate, and U_KV did not change significantly with either infusion. $U_{Na}V$ increased significantly during the second DPH infusion.

Discussion

Several recent studies have suggested that renal sympathetic nerves play an important role in the release of renin. Thus, electrical stimulation of the renal nerves at a level that does not alter renal hemodynamics or urinary sodium excretion is associated with an increase in renin release (4). This effect is blocked by prior administration of the β_1 adrenoceptor antagonists atenolol (4) or metoprolol (5). In contrast, β_2 adrenoceptor antagonists have no effect (4, 6). These findings suggest that renal nerve-stimulated renin release is mediated by β_1 adrenoceptors located on the juxtaglomerular granular cells.

Several investigators have provided evidence of support for a β_1 adrenoceptor mechanism for mediation of renin release in dogs (4, 5, 9) and in rat kidney cortical slices (10, 11). However, others have provided evidence for a β_2 adrenoceptor mechanism in mediating renin release in rabbits (12), dogs (13), and cats (14). Still others have maintained that both β_1 and β_2 adrenoceptors are involved (15, 16). Several factors may have been responsible for this discrepancy. These in-

clude animal species differences, the differences in the methods used to stimulate renin release (isoproterenol administration vs renal nerve stimulation), and differences in the preparations used (intact animal vs kidney slices vs isolated kidneys). In addition, some of the results may have been modified by the activation of non-β adrenergic mediators of renin release such as the baroreceptor or the macula densa.

Previous studies suggested that the effect of DPH on renin release was mediated by, or required, the presence of the renal nerves since renal denervation completely abolished DPH-stimulated renin release (2). In the present study, with the renal nerves intact, we have demonstrated that prior treatment with the β_1 adrenoceptor antagonists, metoprolol or atenolol, abolished DPH-stimulated renin release. Prior treatment with the β_2 adrenoceptor antagonist ICI 118,551 had no effect on DPH-stimulated renin release. These findings suggest that DPH-stimulated renin release is mediated by β_1 adrenoceptors. In this study, each animal served as its own control by receiving a DPH infusion twice. In addition, in the control group, we demonstrated that DPH-stimulated renin release occurred twice. Thus, the lack of renin release in the β_1 antagonists' treated animals was not due to the tachyphylaxis to DPH. In the present study, the changes in systemic blood pressure and urinary sodium excretion were in the same direction in all groups during DPH-adrenergic blocker infusions and were similar to those reported previously in the denervated kidney (2). Thus, changes in systemic hemodynamics and sodium excretion were unlikely to be responsible for the observed alteration of renin release. In this study, renal blood flow did not increase during DPH-metoprolol infusion. However, renal blood flow did increase during the DPH-atenolol infusion although it did not reach statistical significance. Thus, the participation of vascular receptors in the mediation of DPH-stimulated renin release could not be entirely excluded.

The possible role of α adrenoceptors in DPH-stimulated renin release was further explored in this study. Several studies have suggested that α adrenoceptors may be important in the neural control of renin release. Clonidine, an α_2 adrenoceptor agonist has been shown to decrease renin release (17, 18). Other studies have suggested that stimulation of the α_1 adrenoceptors inhibits renin release from kidney slices (19, 20) and from the isolated perfused kidney (21). On the other hand, *in vivo* studies have demonstrated that activation of α_1 adrenoceptors stimulates renin release (22–24). However, this is thought to be due to secondary changes in renal blood flow and sodium excretion. Although Blair (25) has shown that phenylephrine (α_1 agonist) increases renin release at a dose that does not alter renal hemodynamics and sodium excretion, this study was done under the condition of vasodilation associated with aortic clamping. Osborn *et al.* (7) have recently shown that renal α receptor blockade with phentolamine does not affect renin release in response to renal nerve stimulation at a level that does not change renal hemodynamics and sodium excretion. Taken together, these data suggest that α adrenoceptor stimulation that results in a reduction of renal blood flow and sodium excretion increases renin release. In contract, in the absence of renal hemodynamic and sodium excretion changes, α adrenoceptor stimulation does not increase renin release. In this study, blockade of α adrenoceptors by phentolamine did not abolish DPH-stimulated renin release. The dose of phentolamine used in this study is about four times that used by Osborn *et al.* (7). This result suggests that DPH-stimulated renin release is not due to stimulation of α adrenoceptors.

Taken together, the data from this study suggest that DPH-stimulated renin release is mediated by renal β_1 adrenoceptors since the increased renin release is blocked by α_1 antagonists but not by β_2 or α adrenergic antagonist.

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