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Effects of Dibutyryl Cyclic AMP and Propranolol on Renin Secretion in Dogs (39909)

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Introduction. The secretion of renin seems to be regulated, at least in part, by the sympathetic nervous system. Such a regulation appears to involve the β-adrenergic receptors, since the renin secretion provoked by catecholamine infusion or sympathetic nerve stimulation in dogs can be abolished by β-adrenergic blocking agents (1-3).

The relationship between β-adrenergic receptors and the adenylate cyclase system has been well recognized. The stimulation of the β receptor is associated with an increased formation of cyclic AMP (cAMP), and this effect is specifically antagonized by β-adrenergic blocking agents (4).

If the β receptor-adenylate cyclase system is involved in the control of renin secretion, the administration of cAMP may be expected to increase renin secretion. Michelakis *et al.* (5) reported that cAMP increases renin release in renal cell suspension *in vitro*. Winer *et al.* (6) also demonstrated that the infusion of cAMP into the renal artery increases renin secretion in anesthetized dogs, and that such an increase in renin secretion was blocked by propranolol. On the contrary, Tagawa and Vander (7) and Allison *et al.* (8) failed to observe similar effects of cAMP secretion. The latter authors reported that dibutyryl cyclic AMP (DbcAMP) increases arterial plasma renin activity (PRA), along with a decrease in potassium concentration and in urinary sodium excretion.

In view of this conflicting data and because of the importance of establishing a relationship between cAMP and renin secretion, the present study was undertaken. The effects of cAMP and DbcAMP on renin secretion and renal function were studied in anesthetized dogs. The effects of β-adrenergic blocking agents on the DbcAMP-induced renin secretion were also investigated.

The infusion of DbcAMP, but not cAMP, into the renal artery caused a significant increase in renin secretion which appears to be unrelated to the drug-induced changes in renal hemodynamics. DbcAMP-induced renin secretion was not antagonized by β-adrenergic blocking agents.

Methods. Adult mongrel dogs, average body weight 18 kg, were anesthetized with intravenous sodium pentobarbital (30 mg/kg) and mechanically ventilated. The left kidney was exposed through a retroperitoneal flank incision, and denervated as described previously (14). The renal blood flow (RBF) was measured with an electromagnetic flow meter (Nihon Koden, Model MF-25). Arterial blood samples were collected from the axillary artery, and renal venous samples via a catheter introduced through the left spermatic or ovarian vein. Urine was collected from the left ureter. Each clearance period was 10 to 15 min and blood samples were collected in the middle of each clearance period. A 23-gauge needle was introduced into the left renal artery proximal to the flow probe, and saline or drug solution was infused through this needle at a rate of 0.5 ml/min. Glomerular filtration rate (GFR) was measured by creatinine clearance. Electrolytes in plasma and urine were analyzed by a flame photometer (Instrumentation Laboratory, Model 143).

PRA was determined by radioimmunoassay (9) and is expressed as the amount of generated angiotensin I per milliliter of plasma during a 3-hr incubation period (nanograms per milliliter). Renin secretion rate (RSR) was calculated as follows: (renal plasma flow) × (renal venous PRA–renal arterial PRA).

The experiment was divided into two

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parts as follows:

Part 1. Thirteen dogs were divided into two groups, five in cAMP and eight in DbcAMP infusion groups, respectively. cAMP or DbcAMP was infused into the renal artery at a rate of 0.3 mg/kg/min for the total period of 60 min. Two 15-min urine collections, with arterial and renal venous blood samples withdrawn at midpoint of each urine collection period, were taken before the infusion. During the infusion, urine and blood samples were taken at 10, 30, and 60 min. After cessation of infusion, samples were taken at 30 and 60 min.

Part 2. Six dogs were injected iv (axillary vein) with propranolol: 0.6 mg/kg initially, followed by infusion of a maintenance dose of 0.3 mg/kg/hr. Two additional dogs were injected with sotalol: 7.0 mg/kg initially, followed by a maintenance dose of 7.0 mg/kg/hr. Thirty minutes after the injection of β-adrenergic blocking agents, DbcAMP was infused at a rate of 3.0 mg/kg/min into the renal artery for 60 min. Blood and urine samples were collected at 10, 30, and 60 min during the infusion, and two samples were taken at 30 and 60 min after the infusion.

Statistical significance was determined by Student's paired and nonpaired *t* tests. The drugs used were obtained from the following sources: cAMP (Seishin Pharmaceutical Company), DbcAMP (Daiichi Pharmaceutical Company), DL-propranolol (Imperial Chemical Industries), and DL-sotalol (Mead-Johnson Company).

Results. Effect of cAMP and DbcAMP on renal function and renin secretion. The infusion of cAMP into the renal artery at a rate of 0.3 mg/kg/min resulted in an increase in RBF. GFR was transiently decreased at 10 min ($P < 0.05$), but arterial or renal venous PRA was not altered significantly (Table I).

The infusion of DbcAMP into the renal artery at a rate of 0.3 mg/kg/min resulted in a gradual increase in RBF which reached a peak (62% above the control value) at 30 min without any change in renal BP (Table II). The effect of DbcAMP on RBF was greater than that of cAMP at the same dose. GFR was increased by DbcAMP approximately 40% at 30 min ($P < 0.05$). The urine flow and urinary sodium excretion were also increased at 30 and 60 min, reaching maximum at 60 min. RBF, GFR, urine flow, and urinary sodium excretion returned to preinfusion levels at 60 min after the cessation of DbcAMP infusion.

Renal venous PRA was significantly increased by DbcAMP infusion at 10 and 30 min (Table II). Arterial PRA was also increased significantly. After 60 min of DbcAMP infusion, arterial PRA was further increased, but renal venous PRA remained unchanged. RSR increased during the infusion of DbcAMP. After the cessation of DbcAMP infusion, renal venous PRA slowly decreased to the preinfusion level, but arterial PRA remained elevated (Table III).

The effects of cAMP and DbcAMP on renin secretion rate are shown in Fig. 1, which clearly demonstrates that DbcAMP infusion causes a marked increase in the

TABLE I. EFFECTS OF CYCLIC AMP ON RENAL FUNCTION AND RENIN SECRETION.[a]

Time (min)	Renal arterial BP (mm Hg)	Renal blood flow (ml/g·min)	Glomerular filtration rate (ml/g·min)	Urine flow (μl/g·min)	Urinary sodium excretion (μequiv/g·min)	Plasma renin activity (ng/ml)	
						A[b]	V
0	133±3	2.92±0.28	0.69±0.08	9.9±2.3	1.45±0.43	14.6±3.2	15.0±3.2
			cAMP infusion into the renal artery at a rate of 0.3 mg/kg/min				
10	131±3	3.40±0.27*	0.43±0.02*	6.8±1.6	1.04±0.35	11.7±3.3	17.7±2.8
30	130±3	3.59±0.18*	0.56±0.11	5.8±1.6	0.91±0.32	21.6±5.3	25.3±4.7
60	130±3	3.45±0.21*	0.64±0.08	8.0±1.9	1.13±0.32	21.8±2.8	23.6±5.1
			Termination of cAMP infusion				
90	129±3	3.19±0.18	0.68±0.07	11.5±4.8	1.45±0.48	19.0±5.1	22.7±6.7
120	131±2	3.14±0.26	0.71±0.09	12.6±4.1	1.60±0.46	20.3±7.1	19.8±6.4

[a] All values are means ± SE ($N = 5$).
[b] A, Systemic arterial blood; V, renal venous blood.
* Significantly different from corresponding values prior to the cAMP infusion ($P < 0.05$).

TABLE II. EFFECTS OF DIBUTYRYL CYCLIC AMP ON RENAL FUNCTION AND RENIN SECRETION.[a]

Time (min)	Renal arterial BP (mm Hg)	Renal blood flow (ml/g·min)	Glomerular filtration rate (ml/g·min)	Urine flow (μl/g·min)	Urinary sodium excretion (μequiv/g·min)	Plasma renin activity (ng/ml) A[b]	Plasma renin activity (ng/ml) V
0	137±4	3.15±0.13	0.64±0.06	16.9±6.1	3.22±1.29	9.4±4.1	10.6±4.1
DbcAMP infusion into the renal artery at a rate of 0.3 mg/kg/min							
10	134±4	3.79±0.22**	0.76±0.06	34.7±9.7	6.24±1.93*	14.8±4.5*	22.5±6.1*
30	133±4	5.10±0.38**	0.89±0.09*	76.6±25.9*	9.45±3.05*	24.6±6.3**	55.3±12.6**
60	133±6	4.70±0.26*	0.87±0.12	90.3±26.4*	14.02±4.39*	32.6±9.7**	52.3±17.3*
Termination of DbcAMP infusion							
90	133±4	3.60±0.24	0.76±0.07	31.8±9.5*	5.99±1.85*	36.8±12.0*	50.4±20.3
120	134±4	3.39±0.20	0.73±0.05	22.3±6.9	4.05±1.32	38.7±15.5	45.7±19.5

[a] All values are means ± SE (N = 8).
[b] A, Systemic arterial blood; V, renal venous blood.
* Significantly different from corresponding value observed prior to DbcAMP infusion ($P < 0.05$).
**Significantly different from corresponding value observed prior to DbcAMP infusion ($P < 0.01$).

TABLE III. EFFECTS OF INTRAVENOUS ADMINISTRATION OF β-ADRENERGIC BLOCKING AGENTS AND DIBUTYRL CYCLIC AMP ON RENAL FUNCTION AND RENIN SECRETION.

	Renal arterial BP	Renal blood flow	Glomerular filtration rate	Urine flow	Urine sodium excretion	Plasma renin activity A[a]	Plasma renin activity V
Control	122±2	3.13±0.09	0.61±0.03	10.4±2.2	2.07±0.31	11.8±2.8	14.3±2.5
β-Adrenergic blockade[b]	108±4**	3.14±0.22	0.42±0.03**	12.5±3.4	2.23±0.56	7.5±1.6*	8.1±1.7*
β-Adrenergic blockade[c] plus DbcAMP (30 min)	108±4	5.20±0.37****	0.58±0.05***	31.2±9.2***	4.25±0.88****	23.0±6.9	52.3±13.7***
β-Adrenergic blockade[c] plus DbcAMP (60 min)	110±4	5.33±0.41****	0.57±0.05	42.1±12.1***	5.23±1.25****	31.0±11.3***	48.5±13.1***

[a] A, Systemic arterial blood; V, renal venous blood.
[b] Propranolol (0.6 mg/kg) or sotalol (7 mg/kg) was administered intravenously.
[c] After the initial dose of propranolol or sotalol, maintenance dose of these agents was infused continuously.
* Significantly different from values observed prior to the administration of β-adrenergic blocking agents ($P < 0.05$).
** Significantly different from values observed prior to the administration of β-adrenergic blocking agents ($P < 0.01$).
*** Significantly different from values observed in the presence of β-adrenergic blocking agents, but prior to the administration of DbcAMP ($P < 0.05$).
**** Significantly different from values observed in the presence of β-adrenergic blocking agents, but prior to the administration of DbcAMP ($P < 0.01$).

renin secretion rate. In contrast, cAMP-induced changes in renin secretion rate were rather minimal. Due to the relatively large variability of observed values, changes in renin secretion rate due to cAMP infusion were statistically not significant. The maximal renin secretion induced by DbcAMP occurred after 30 min of the infusion (Fig. 1), but the maximal effects of DbcAMP on urine flow and sodium excretion were found after 60 min of the infusion (Table II).

Plasma sodium and potassium concentrations were not changed significantly during the DbcAMP infusion. Plasma sodium and potassium concentrations prior to the infusion were 138.1 ± 3.3 and 3.4 ± 0.1 mequiv/liter, respectively. These values after the 30 min of DbcAMP infusion were 139.3 ± 3.9 and 3.2 ± 0.1 mequiv/liter, respectively.

Effects of propranolol and sotalol on renal function and renin secretion. The effects of propranolol and sotalol on renal function and renin secretion are shown in Table III. Both β-adrenergic blockers, propranolol and sotalol, had similar effects on renal function and renin secretion. Systemic BP was promptly decreased immediately after the injection of propranolol (0.6 mg/kg) or sotalol (7.0 mg/kg), and was stabilized at 108 ± 4 mm Hg. RBF and urinary sodium excretion were not changed, but GFR was significantly decreased ($P < 0.01$). Both arterial and renal venous PRA were significantly decreased at 30 min after the injection of β blocking agents ($P < 0.05$). RSR

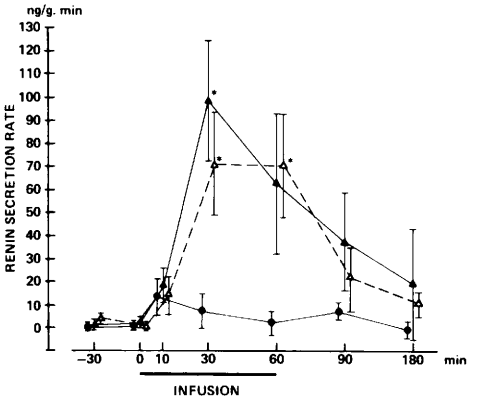

FIG. 1. Renin secretion rate during renal arterial infusion of cyclic AMP or dibutylyl cyclic AMP in the absence or presence of propranolol. After a control observation period, either cAMP or DbcAMP was infused into the left renal artery of anesthetized dogs at a rate of 0.3 mg/kg/min for 60 min (indicated by the bar). Filled circles: cAMP (mean of five experiments). Filled triangles: DbcAMP in the absence of propranolol (mean of eight experiments). Open triangles: DbcAMP in the presence of propranolol (mean of six experiments; propranolol, 0.6 mg/kg, was injected intravenously 30 min before the initiation of DbcAMP infusion). Vertical lines indicate SE. Asterisks indicate significant changes compared to control values observed immediately before the drug infusion (0 min).

was also decreased by β-adrenergic blocking agents ($P < 0.05$).

During such an infusion, initial administration of propranolol or sotalol was followed by an additional infusion of maintenance dose of these agents. DbcAMP was infused into the renal artery at a rate of 0.3 mg/kg/min. DbcAMP increased both arterial and renal venous PRA and RSR in the presence of the β-adrenergic blocking agent. RBF, GFR, and urinary sodium excretion were also increased by the DbcAMP infusion, although changes in these parameters were smaller in the presence of β-adrenergic blocking agent.

The effects of DbcAMP on renin secretion with and without propranolol treatment are compared in Fig. 1. Propranolol treatment failed to affect the DbcAMP-induced renin secretion: Both the time course and magnitude of the increase in renin secretion were unchanged.

Discussion. Our results show that the infusion of DbcAMP into the renal artery at a rate of 0.3 mg/kg/min increased renin secretion in anesthetized dogs, and confirm an earlier report by Allison *et al.* (8). These investigators assayed arterial PRA only, and reported a significant increase in PRA at 30 min after the cessation of DbcAMP infusion. In the present study, both arterial and renal venous PRA were estimated simultaneously and RSR was calculated. As shown in Table II, renal venous PRA increased gradually during the infusion of DbcAMP, reached a maximum at 30 min, and then remained at the elevated level.

In general, an increase in intracellular cAMP of smooth muscle cells is associated with the relaxation of the muscle (10). In the present study, the administration of cAMP or DbcAMP caused a dilation of the renal vessels. In addition, DbcAMP increased the urine flow and urinary sodium excretion, confirming a previous report (11). It is well known that these changes in renal hemodynamics and sodium metabolism could influence the renin secretion (12–14). However, any significant interrelationship between changes in RSR and RBF or urinary sodium excretion was not observed.

The maximum response in renin secretion to DbcAMP preceded the maximum response in urinary sodium excretion. Allison *et al.* (8) suggested that an increase in arterial PRA is the result of a decreased plasma potassium concentration. In the present study, however, DbcAMP increased RSR without changes in plasma potassium concentration throughout the experiment. Thus, the renin secretion seems to be induced via mechanisms other than changes in renal hemodynamics and electrolytes metabolism.

A number of investigators suggested that the β-adrenergic mechanism plays an important role in renin secretion (1, 2, 15–17). The present results, showing that the β-adrenergic blocking agents propranolol and sotalol are capable of decreasing both arterial and renal venous PRA, are consistent with the contention that the β-adrenergic mechanism is involved in renin secretion. It is generally accepted that the stimulation of β-adrenergic receptors activates an adenylate cyclase and increases intracellular cAMP concentration in various tissues, including the kidney (4, 18). Thus, the β-adrenergic system may play a role in the

regulation of renin release through cAMP concentration in the kidney.

Winer *et al.* (6) reported that a small amount of exogenously administered cAMP increased renin release. Tagawa and Vander (7), however, failed to observe such an effect. Reid *et al.* (19) reported that theophylline, which increases intracellular levels of cAMP by inhibition of phosphodiesterase, stimulates renin secretion. In the present study, cAMP failed to cause a significant increase in renin secretion. Although the possibility that cAMP increases the renin secretion could not be completely ruled out in the present study, cAMP-induced changes, if any, are markedly smaller than those caused by DbcAMP, when both nucleotides were infused at the same infusion rate. The difference in action of cAMP and DbcAMP may be explained by the previous findings that DbcAMP would accumulate in cells to a sufficient level to exert an effect on renin secretion, whereas cAMP is relatively impermeable through the cell membrane (20). However, it is still not clear whether DbcAMP stimulates renin secretion directly or after conversion to cAMP in the juxtaglomerular cells (JGC). Winer *et al.* (6) reported that an infusion of cAMP causes a marked increase in renin secretion, and that the effect of cAMP was blocked by α- or β-adrenergic blocking agents. It was suggested that these agents suppressed renin secretion at a step which follows cAMP production, rather than that which precedes cAMP production, such as adrenergic receptors on cytoplasmic membranes or the inhibition of adenylate cyclase. In the present study, however, the stimulatory effect of DbcAMP on renin secretion was not modified by the adrenergic blocking agents propranolol or sotalol. The latter compound lacks quinidine-like membrane stabilizing effects. The cause of apparent discrepancies between the present data and those reported by Winer *et al.* (6) is unclear.

Recently, Beck *et al.* (21) reported that isoproterenol enhances renin release in lithium-treated dogs, although isoproterenol failed to stimulate the generation of cAMP under these experimental conditions. Thus, it appears possible that isoproterenol may be capable of enhancing renin secretion by another mechanism, in addition to causing renin secretion by increasing cellular cAMP concentrations. The present findings extend our previous observation *in vitro* that DbcAMP stimulates renin release from renal cortex and isolated renin granules (22). It would seem that the action of DbcAMP is mainly on the renin release mechanism in JGC. However, cAMP and DbcAMP have complex actions on membrane permeability, protein kinase activity, and calcium metabolism (23). Further experiments seem necessary to clarify the relationship between cyclic nucleotides and renin secretion.

Summary. The effect of cAMP and DbcAMP on renin secretion and renal hemodynamics was studied in anesthetized dogs. The infusion of cAMP into a renal artery failed to increase renin secretion significantly, but DbcAMP caused a significant increase in renin secretion. Both cAMP and DbcAMP had a vasodilator effect on renal vessels, without any change in renal blood pressure. These two compounds, however, differed in their effects on GFR and urine flow. cAMP infusion transiently decreased GFR, without significant effects on urinary flow and urinary sodium excretion, but DbcAMP increased GFR, urinary flow, and urinary sodium excretion. Time course studies, however, indicated that there was no direct relationship between renin secretion and RBF or urinary sodium excretion during DbcAMP infusion. The β-adrenergic blocking agents, propranolol and sotalol, decreased both arterial and renal venous PRA, but failed to affect the DbcAMP-induced renin secretion.

These findings suggest that the effect of DbcAMP on renin secretion is associated with its action on JGC and not with electrolyte or renal hemodynamic changes.

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