Biochemical Pharmacology, Vol. 29, pp. 1933–1937

0006-2952/80/0701-1933 $02.00/0

LACK OF EFFECT OF CHOLINERGIC AGENTS OR ENDOGENOUS GUANOSINE 3′:5′-MONOPHOSPHATE ON RENIN RELEASE BY SLICES OF RAT RENAL CORTEX *IN VITRO** †

STEVEN B. LEICHTER, THEODORE A. KOTCHEN, W. ALLEN RADER and JERRY RADER
Division of Endocrinology, Department of Medicine, University of Kentucky College of Medicine, Lexington, KY 40536, U.S.A.

(*Received* 15 *November* 1979; *accepted* 21 *January* 1980)

Abstract—Cyclic GMP, in concentrations exceeding 50 μM, and cyclic AMP, in concentrations exceeding 1 μM, significantly increased the release of renin by slices of rat renal cortex. Stimulants of cyclic AMP accumulation, such as epinephrine or norepinephrine, 1 μM, produced a significant increase of renin release but did not alter cyclic GMP levels. In contrast, acetylcholine, carbamylcholine, bethanachol, methachol and NaN_3 increased cyclic GMP accumulation without modifying renin release. These results suggest that exogenous cyclic GMP acts as a weak agonist of cyclic AMP on renin release in renal cortex. The effects observed with exogenous cyclic GMP, however, may not represent a physiological action since various stimulants of endogenous cyclic GMP accumulation did not alter renin secretion.

Cyclic AMP stimulates renin release by rat kidney [1–3], and may mediate the stimulatory effects of catecholamines on renin release [1, 2, 4–6]. The effect of cyclic GMP on renin secretion has not been investigated in detail. In the present study, we examined the effects of cyclic GMP and stimulants of cyclic GMP accumulation on renin release by slices of rat renal cortex.

MATERIALS AND METHODS

Female Sprague–Dawley rats, 200–250 g, were killed by cervical fracture. The kidneys were removed and placed in ice-cold 0.25 M sucrose. Following bisection, cortices were separated from medullae. Slices of superficial renal cortex, 40–60 mg, were prepared with a Stadie–Riggs microtome.

Slices were incubated at 37° in a 95% O_2–5% CO_2 atmosphere in Krebs–Ringer bicarbonate buffer [7]. Bovine serum albumin (Fraction V) (250 mg/100 ml), dextrose (200 mg/100 ml), and where indicated, theophylline (180 mg/100 ml) were added. The buffer was adjusted to 300 mOsmoles/l by the addition of mannitol. Pilot experiments demonstrated that renin release by slices incubated in this buffer was equal to or greater than release by slices incubated in Robinson's medium [8]. The O_2–CO_2 atmosphere was introduced over the incubates, at a partial pressure of 10 mm Hg, rather than bubbled through the medium [9]. After 30 min, the slices were placed in 2.1 ml of fresh buffer, containing test substances or their diluents, and were incubated for 2–15 min in a metabolic incubator.

Assay of renin release by renal cortical slices. Incubations were terminated after 15 or 30 min by removing the slices and placing the vials with medium in an ice–water slurry. A 100-μl aliquot of incubate was added to 900 μl of renin substrate (1000 ng/ml) prepared from plasma of anephric sheep [10]. To maintain constant pH, 0.1 ml phosphate buffer (pH 7.4) was added. Angiotensinases and converting enzyme were inhibited by addition of 8-hydroxyquinoline (10 μl of a 0.34 M solution/ml), dimercaptopropanol (10 μl of a 2% solution/ml) and neomycin sulfate. The mixture was incubated for 60 min at 37°. The incubations were terminated by immersion in an ice–water slurry. Radioimmunoassay for angiotensin I (AI) was carried out with 10- and 50-μl aliquots of these incubates according to the method of Haber *et al.* [11]. Results are expressed as ng AI/ml/30 min/g wet tissue weight, in order to facilitate comparison with previous studies [1, 3, 4]. Pilot experiments demonstrated that neither Krebs buffer nor Robinson's medium, incubated without tissue slices, contained any detectable renin activity in this assay. In addition, none of the test substances added to these media in the experiments presented below altered the generation of AI in this system.

Studies on cyclic nucleotide accumulation. Slices of renal cortex were preincubated for 15 min, and incubated for 2 min, as described above. The incubations were terminated, and cyclic nucleotides were extracted by our modifications [12, 13] of the method of Chase [14]. Tissue slices were removed from the incubates, quick-frozen in liquid nitrogen, and boiled for 10 min in 0.05 M acetate buffer, pH 6.25. The slices were homogenized, centrifuged for 30 min at 10,000 *g*, again quick-frozen, thawed, and centrifuged once more at 10,000 *g* for 30 min. Radioimmunoassays for cyclic AMP and cyclic GMP were carried out on the final supernatant fraction by the method of Steiner *et al.* [15] with a commercial kit

* Supported, in part, by a grant from the Kentucky Heart Association.

† Presented, in part, at the Sixty-first Annual Meeting of the Endocrine Society, 13–15 June 1979, Anaheim, CA.

(New England Nuclear, Boston, MA). Samples acetylated before assay [16] yielded the same results as unacetylated samples.

Materials. Cyclic AMP, cyclic GMP, epinephrine, norepinephrine, acetylcholine, hexamethonium, carbamylcholine, bethanachol, methachol, sodium azide, bovine serum albumin (Fraction V), atropine sulfate, cellulose gel and theophylline were all purchased from the Sigma Chemical Co. (St. Louis, MO.). Radioimmunoassay kits for cyclic AMP and GMP were obtained from New England Nuclear. All solutions were prepared fresh daily. The stability of stock acetylcholine and carbamylcholine was tested periodically by thin-layer chromatography with cellulose gel [17].

RESULTS

Effects of exogenous cyclic GMP on renin release. The effects of exogenous cyclic GMP or cyclic AMP (0.1–100 μM) on renin release were compared. Neither cyclic nucleotide at a concentration less than 1 μM increased renin release. Significant stimulation of renin release with cyclic AMP was noted with concentrations exceeding 1 μM (Fig. 1). Concentrations of cyclic GMP exceeding 50 μM were necessary to cause a detectable increase in renin release, although the stimulation by 10 μM cyclic GMP approached statistical significance. The stimulation of renin release by submaximal concentrations of cyclic AMP and cyclic GMP were additive (not shown); however, combinations of maximal stimulatory concentrations of cyclic AMP and GMP were not additive (Fig. 2).

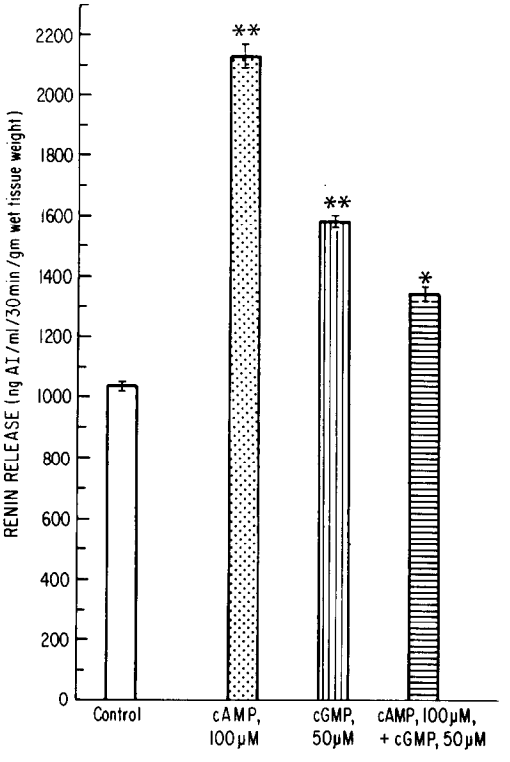

Fig. 2. Effects of exogenous cyclic AMP and exogenous cyclic GMP on renin release by slices of rat renal cortex (N = 16). Key: (*) $P < 0.05$ that the difference between this value and the control value would occur by chance; and (**) $P < 0.01$ that the difference between this value and the control value would occur by chance.

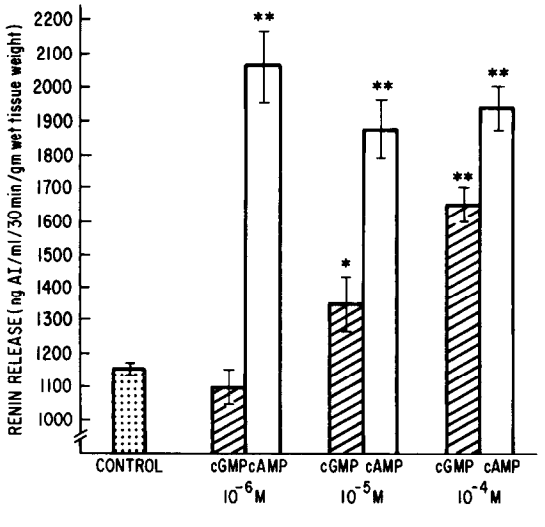

Fig. 1. Effects of increasing concentrations of exogenous cyclic AMP or exogenous cyclic GMP on renin release by slices of rat renal cortex (N = 8). Key: (*) $P < 0.07$ that the difference between this value and the control value would occur by chance; and (**) $P < 0.01$ that the difference between this value and the control value would occur by chance.

Effects of acetylcholine on cyclic GMP accumulation and renin release. We investigated the effects of acetylcholine on both endogenous cyclic GMP accumulation and renin release by slices of rat renal cortex. Acetylcholine (10 nM to 10 μM) caused a significant increase in the levels of cyclic GMP, but failed to alter renin release after 15 min (Fig. 3) or after 30 min of incubation (not shown). The addition of 1 μM eserine sulfate alone or with acetylcholine was without effect on renin release (not shown). The stimulation of cyclic GMP accumulation by acetylcholine was abolished by 1 μM atropine sulfate, but not by 1 μM hexamethonium (Fig. 4).

Effects of other cholinergic agents on cyclic GMP accumulation and renin release. The effects of carbachol, methachol and bethanachol on cyclic GMP accumulation and renin release by slices of rat renal cortex were tested. As with acetylcholine, 10 nM or 10 μM bethanachol increased the concentration of cyclic GMP in these slices from 47 ± 1 to 138 ± 3 or 185 ± 5 pmoles/g tissue, respectively; however, renin release was unaffected. Carbachol and methachol also increased cyclic GMP accumulation without altering renin release (not shown).

Effects of catecholamines on cyclic nucleotide accumulation and renin release. In contrast to cholinergic agents, both epinephrine and norepinephrine (10 nM to 10 μM) stimulated renin release by slices

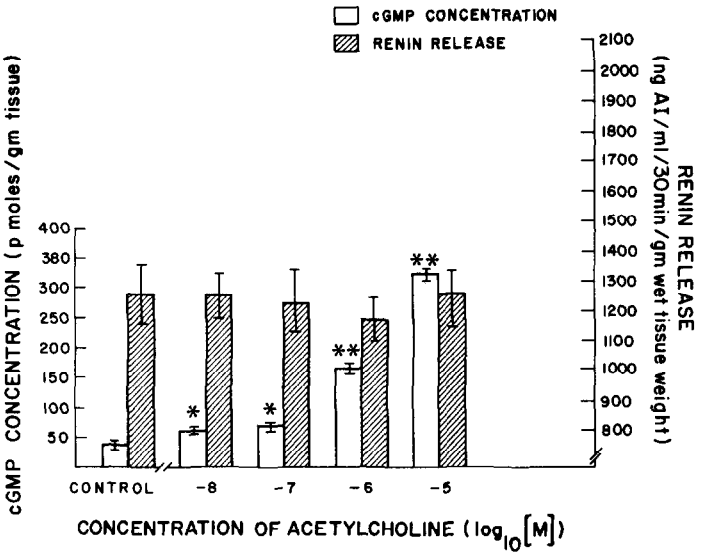

Fig. 3. Effects of acetylcholine on cyclic GMP accumulation and renin release by slices of rat renal cortex (N = 16). Key: (*) $P < 0.05$ that the difference between this value and the control value would occur by chance; and (**) $P < 0.001$ that the difference between this value and the control value would occur by chance.

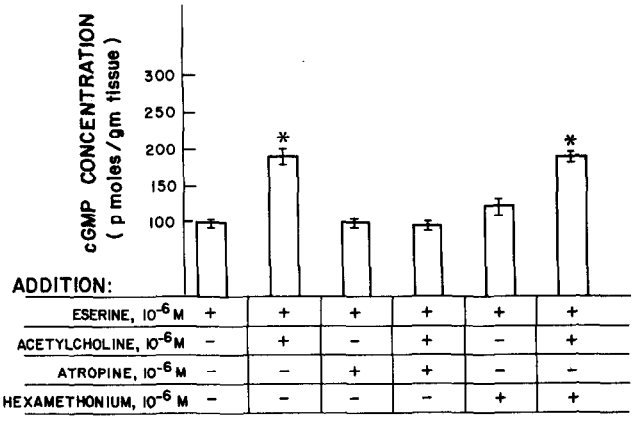

Fig. 4. Cyclic GMP accumulation in slices of rat renal cortex (N = 6). Key: (*) $P < 0.01$ that the difference between this value and the control value would occur by chance.

Table 1. Renin release by slices of rat renal cortex *in vitro*

Addition	Renin release (ng AI/ml/30 min/g wet tissue wt)	No. of slices	P
None	1074 ± 7	40	
Epinephrine (10^{-6} M)	2515 ± 34	23	< 0.01
Norepinephrine (10^{-6} M)	1994 ± 30	23	< 0.01

Table 2. Cyclic nucleotide concentration in slices of rat renal cortex*

Addition	Cyclic nucleotide concentration (pmoles/g wet tissue wt) cAMP	cGMP
None	1155 ± 35 (47)†	42.5 ± 1.9 (35)
Norepinephrine (10^{-6} M)	2741 ± 244‡ (18)	49.4 ± 2.8 (18)
Epinephrine (10^{-6} M)	1932 ± 133‡ (15)	48.7 ± 4.5 (15)

* Slices (40–60 mg) were preincubated and incubated, as described in the text. Theophylline (180 mg/100 ml) was added to all preincubates and incubates.
† Number of observations.
‡ $P < 0.01$.

of rat renal cortex (Table 1). This increase was associated with an increase in tissue levels of cyclic AMP, but not of cyclic GMP (Table 2).

DISCUSSION

Since cyclic AMP is known to stimulate renin release [1, 3, 4, 18], there was a basis for investigating whether cGMP also has an effect on renin release. Effects of cyclic GMP on renin secretion have been investigated in two previous studies [18, 19]. In one study, the infusion of exogenous cyclic GMP into an isolated, perfused rat kidney was noted to cause a slight increase in the renin content of the perfusate [19]; however, whether this was a direct effect of cyclic GMP on juxtaglomerular cells, or an indirect effect mediated by alterations in renal hemodynamics was unclear. In another study, 8-bromo-c GMP had no effect on renin release [18].

The results of the present study indicate that exogenous cyclic GMP, like cyclic AMP, stimulates renin release by slices of rat renal cortex *in vitro*. Cyclic GMP is a less potent stimulus than cyclic AMP. Concentrations of cyclic AMP as low as 1 μM produced a significant increase in renin release by these slices. In comparison, concentrations of cyclic GMP exceeding 5×10^{-5} M were necessary to produce a significant increase. These data are consistent with previous results in other tissues, such as liver or adipose tissue, showing that exogenous cyclic GMP mimics the effects of cyclic AMP on hepatic glucose metabolism [20–23] or lipolysis [24].

The present study, however, also suggests that effects noted with exogenous cyclic GMP and renin secretion in our system may not be representative of the physiological actions of endogenous cyclic GMP in renal cortex. In contrast to exogenous cyclic GMP, a variety of cholinergic agents or NaN_3, which stimulate endogenous cyclic GMP accumulation, do not alter renin release by renal cortical slices. These effects were noted over a wide concentration range with each substance, and the various concentrations of each agent studied included concentrations which effectively stimulated cyclic GMP accumulation. In addition, these data show that a combination of maximum stimulatory concentrations of exogenous cyclic AMP and cyclic GMP do not have additive effects on renin secretion, suggesting that exogenous cyclic GMP and cyclic AMP may stimulate renin secretion via the same or similar mechanisms. Differential effects of exogenous versus endogenous cyclic GMP have been reported in other systems. Whereas exogenous cyclic GMP stimulates glycogenolysis in liver [12, 20], acetylcholine may cause a stimulation of glycogen synthesis [25]. Similarly, exogenous cyclic GMP inhibits the hepatic L-form of pyruvate kinase activity [23], but cholinergic agents or sodium azide may not modify this enzyme activity [26]. Thus, the present study and these previous reports raise questions about the relevance of studies using exogenous cyclic GMP to investigate the physiological actions of this cyclic nucleotide.

REFERENCES

1. A. M. Michelakis, J. Caudle and G. W. Leddle, *Proc. Soc. exp. Biol. Med.* **130**, 748 (1969).
2. N. Winter, D. S. Chokski and W. G. Walkenhorst, *Circulation Res.* **29**, 239 (1971).
3. K. Gaal, I. Forgacs and Z. Bacsalmasy, *Acta physiol. hung.* **47**, 49 (1976).
4. W. Aoi, M. B. Wade, D. R. Rosner and M. H. Weinberger, *Am. J. Physiol.* **227**, 630 (1974).
5. N. P. Beck, S. W. Reed, H. V. Murdaugh and B. B. Davis, *J. clin. Invest.* **51**, 939 (1972).
6. W. B. Campbell, R. M. Graham and E. K. Jackson, *J. clin. Invest.* **64**, 448 (1979).
7. W. W. Umbreit, *Manometric Techniques*, p. 132. (Burgess Publishing Co., Minneapolis (1964).
8. J. R. Robinson, *Biochem. J.* **45**, 68 (1949).
9. K. W. Cho and R. L. Malvin, *Am. J. Physiol.* **236**, F501 (1979).
10. S. C. Skinner, *Circulation Res.* **20**, 391 (1967).
11. E. Haber, T. Koerner, L. B. Page, B. Kliman and A. Purnode, *J. clin. Endocr. Metab.* **29**, 1349 (1969).
12. S. B. Leichter and L. R. Chase, *Endocrinology* **102**, 785 (1978).
13. S. B. Leichter and P. L. Poffenbarger, *Biochem. biophys. Res. Commun.* **84**, 403 (1978).
14. L. R. Chase, *Endocrinology* **96**, 70 (1975).
15. A. L. Steiner, A. S. Pagliara, L. R. Chase and D. M. Kipnis, *J. biol. Chem.* **247**, 1114 (1972).
16. E. R. Fransden and G. Krishna, *Life Sci.* **18**, 529 (1976).
17. E. Stahl, *Thin Layer Chromatography*. Springer, New York (1968).
18. K. G. Hofbauer, A. Konrads, K. Schwarz and U. Werner, *Klin. Wschr.* **56** (Suppl. 1), 51 (1978).
19. W. S. Peart, T. Quesada and I. Tenyi, *Br. J. Pharmac.* **54**, 55 (1975).
20. K. K. Schlender, S. H. Wei and C. Villar-Palasi, *Biochim. biophys Acta* **191**, 272 (1969).
21. H. O. Conn and D. M. Kipnis, *Biochem. biophys. Res. Commun.* **37**, 319 (1969).

22. T. H. Claus and S. J. Pilkis, *Biochim. biophys. Acta* **421**, 246 (1976).
23. T. J. C. Van Berkel, J. K. Kruitjt, J. F. Koster and W. C. Hulsman, *Biochem. biophys. Res. Commun.* **72**, 917 (1976).
24. T. Braun, O. Hechter and H. P. Bar, *Proc. Soc. exp. Biol. Med.* **132**, 233 (1969).
25. J. O. Akpan, R. Gardner and S. R. Wagle, *Biochem. biophys. Res. Commun.* **61**, 222 (1974).
26. S. Leichter and J. W. Anderson, *Diabetes* **28** (Suppl.), 423 (1979).