Effects of Cyclic AMP and Dibutyryl Cyclic AMP on Renin Release in Vivo and in Vitro

KENJIRO YAMAMOTO, M.D., TAKEHIKO OKAHARA, M.D.,
YUKIOI ABE, M.D., JURO UEDA, M.D.,
TAKESHI KISHIMOTO, M.D., AND SHIRO MORIMOTO, M.D.

Previously we reported that isoproterenol stimulates renin release in the anesthetized dogs and this stimulation is completely blocked by propranolol. These results are similar to the data reported by other investigators. Generally, adrenergic β-stimulants increase the activity of membrane binding adenylcyclase and result in an increase of cyclic AMP (C-AMP) in the target cells. Is this mechanism included in the stimulating action of isoproterenol on renin secretion from the kidney? This possibility is supported by the fact that theophylline, which inhibits phosphodiesterase, stimulates renin release in dogs and humans. Furthermore, several investigators reported that C-AMP and dibutyryl C-AMP (dC-AMP) increased plasma renin activity (PRA) in dogs and rats, but other investigators reported that C-AMP did not stimulate renin release in dogs. Previously, we reported that the effect of C-AMP and dC-AMP on the renal function and renal hemodynamics. In the present experiments, we investigated the effect of both agents on renin release in the anesthetized dogs.

On the other hand, two in vitro experiments were reported from our laboratory; renin release from renal cortical slices, and the isolation of renin granules from renal cortex. In the present experiments, the effects of C-AMP and dC-AMP on the renin release from cortex slices and from isolated renin granules were studied, and the effect of Ca++ on the in vitro renin release was also investigated.

Methods

1) in vivo experiment

Male mongrel dogs, 12–17 kg, were anesthetized with Nembutal in a dose of 30 mg/kg i.v. The left kidney was exposed through a retroperitoneal flank incision, and all visible renal nerves were cut. Renal blood flow (RBF) was measured by an electromagnetic flowmeter (Nihon-Koden MF-25). A polyethylene tube containing heparin-saline was introduced into the left femoral artery, and pushed up to the aorta at the level of the renal artery, and the pressure was monitored by a pressure transducer. Systemic arterial blood samples were collected from the right brachial artery and the renal venous blood was collected via a tube inserted into the left renal vein through the left spermatic or ovarian vein. A No. 23 gauge needle was introduced into the left renal artery proximal to the flow probe and saline (37°C) was infused by an infusion pump at a rate of 0.5 ml/min, and drug solution was also infused by this system. Intravenous infusion of 0.9% saline, 4.2 ml/min, was started after anesthesia. After sugery was completed, a loading dose of creatinine, 100 mg/kg, was given intravenously, followed by a maintenance dose of 50 mg/kg/hr. Glomerular filtration rate (GFR) was calculated as follows: GFR = (Systemic arterial concentration of creatinine − Renal venous concentration of creatinine) / Systemic arterial concentration of creatinine × Renal plasma flow.

A polyethylene tube was inserted into the left ureter and urine was collected throughout the

Key Words:

Renin, Cyclic AMP, Dibutyryl cyclic AMP, Renin granule

Department of Pharmacology and Urology, Osaka City University Medical School, and Osaka College of Pharmacy

* This paper was presented on the II Conference of Pathogenesis of Hypertension, November 20, 1972, Fukuoka

Japanese Circulation Journal Vol. 37, October 1973 1271
Fig. 1. Effect of Intrarenal Arterial Infusion of Dibutyryl C-AMP on the Systemic Arterial PRA and Renin Secretion Rate.

After completion of surgery, three 10-minute control periods were obtained, and then cyclic AMP (C-AMP) or dibutyryl cyclic AMP (dC-AMP) was infused into the renal artery at a rate of 300 μg/kg/min. During drug infusion, arterial and renal venous blood samples were withdrawn every 10 minutes and, two more samples were obtained 30 and 60 minutes after stop the drug infusion. Plasma was separated immediately in a cold room and kept in deep freezer. Plasma renin activity (PRA) was measured by radioimmunoassay and sodium and potassium of plasma and urine were determined flame photometrically. Osmolarity of plasma and urine was determined by Fiske Osmometer.

II) In vitro experiment
A) Renin release from renal cortical slices

The experiment was performed as described in previous papers. Briefly, 200 mg of renal cortical slices was incubated with 10 ml of Krebs' bicarbonate buffer solution (pH 7.2–7.3) containing glucose (200 mg%) at 25°C. One ml of the medium was taken at 30', 60' and 120' after the start of incubation and cooled immediately, and cell debris was removed by centrifugation. Renin content of the supernatant was determined after reincubation with renin substrate. Renin release was expressed as angiotensin equivalents ng/ml medium at different time of incubation.

C-AMP and dC-AMP were added to the incubation medium at a concentration from $10^{-4}$ M to $10^{-8}$ M. In some cases, calcium was omitted from the Krebs' buffer.

B) Renin release from the isolated renin granules

Renal cortex was gently homogenized with cold 0.45 M sucrose solution, and after removing the cell debris and cell nuclei by low speed centrifugation, the obtained supernatant was served for sucrose density gradient centrifugation. After 90' centrifugation at 60,000 x g, renin granule rich fraction was equilibrated with 1.6 M sucrose solution (Fraction 2). This fraction was used for the following experiments. To 0.4 ml of this fraction 1.6 ml of Krebs' bicarbonate buffer (pH 7.2–7.3) was added and incubated for 15' at 37°C. The incubation mixture was immediately cooled and renin granules were spun down by centrifugation at 100,000 x g for 30 minutes. After removing the supernatant, 0.1%
Fig. 2. Effect of Dibutyryl C-AMP on Renin Release from Renal Cortex Slices.

Fig. 3. Effect of Dibutyryl C-AMP, C-AMP and Ca\(^{2+}\) Free on the Renin Release from Isolated Renin Granules.

Triton X-100 was added to the pellet and mixed vigorously. Renin activities of the supernatant and the pellet were determined.

Renin activity was determined as follows; 0.2 ml of the medium was preincubated with 0.4 ml of 0.1 M phosphate buffer (pH 7.0) with
EDTA (10^{-2} M), 8-hydroxyquinoline (6.6%, 0.01 ml) and dimercaprol (10%, 0.002 ml) at 37°C for 10'. The reaction was started by the addition of 0.4 ml of purified dog renin substrate, and performed incubation for 2 hrs. After the incubation, generated angiotensin I was measured by radioimmunoassay.

Renin substrate used in this experiment was prepared from nephrectomized dog plasma as described in a previous paper. It contained at least 1600 ng angiotensin I equivalents per ml and practically no renin activity.

RESULTS AND DISCUSSION

I) In vivo experiment

Renal venous PRA was increased by an infusion of dC-AMP accompanied with an increase in systemic arterial PRA. Renin secretion rate [(Renal venous PRA - Systemic arterial PRA) x Renal plasma flow] was also increased by dC-AMP (Fig. 1). These data are similar to those reported by Allison et al in dogs and Hauger-Klevene et al in rats. After 50 to 60 minutes, even though the infusion of dC-AMP was continued, once elevated renal venous PRA was decreased, accompanied with continued increase in systemic arterial PRA. We did not determine renal renin content after the experiment, but generally, renin content in the kidney is considerably higher than the released renin. Probably, dC-AMP has an inhibitory action on the renin release. This assumption will be backed up by an in vitro experiment described below, but we cannot exclude a possibility that some renin inhibitors are released from the kidney by dC-AMP.

Infusion of dC-AMP resulted in a significant increase of RBF, UF, Na excretion and also free water reabsorption. These parameters reached the maximum 30 to 40 minutes after the infusion and continued during the infusion, and all those parameters are gradually returned to the control level after cessation of the infusion. Since dC-AMP increases RBF and Na excretion without any change in systemic arterial pressure, the stimulating action on renin release is not explained by the classical baroreceptor or macula densa theory.

Following infusion of C-AMP, renal venous PRA and RSR were increased, but statistically insignificant. RBF was also slightly increased, and UF and Na excretion were slightly decreased by C-AMP.

II) In vitro experiment

A) Renin release from renal cortex slices

Renin was released from the renal cortex slices to the incubation medium containing Ca^{++}, while renin release was markedly suppressed throughout the incubation period in Ca^{+} free medium. dC AMP (10^{-2} M - 10^{-5} M) showed a biphasic effect on renin release from the renal cortex slices; it stimulates about 20% at 30' incubation, and supresses about 20% at 120' incubation (Fig. 2). This result supports the effect of dC-AMP on the renin release in vivo described above. Michelakis et al found that C-AMP stimulates renin release and production in vitro using renal cell suspension. In the present experiments, C-AMP (10^{-3} M - 10^{-5} M) stimulated renin release from the renal cortex slices at 30' and 60' incubation, but insignificantly.

B) Renin release from the isolated renin granules

When renin granule fraction was incubated with Krebs' bicarbonate buffer at 37°C, about 20% of renin was released from renin granules into the medium during 15'. This renin release was not influenced by omitting Ca^{++} from the medium. C-AMP (10^{-3} M - 10^{-5} M) stimulated renin release slightly, however, dC-AMP stimulated markedly at the same concentrations (Fig. 3).

Conclusion

1) Infusion of dibutyryl C-AMP (300 μg/kg.min) increased renin release, however, once increased PRA of the renal venous blood from the infused kidney decreased during the continuous infusion. Renin release was also increased by C-AMP (300 μg/kg.min), but not significantly.

2) Ca^{++} was essential for renin release from renal cortex slices. Dibutyryl C-AMP (10^{-4} M - 10^{-5} M) stimulated renin release with short time incubation, but inhibited with prolonged incubation. C-AMP (10^{-3} M - 10^{-5} M) showed a tendency to stimulate renin release, but statistically insignificant.

3) Ca^{++} was not essential for renin release from the isolated renin granules. Dibutyryl C-AMP (10^{-3} M-10^{-5} M) stimulated renin release from the granules, but C-AMP (10^{-3} M - 10^{-5} M) did not.

REFERENCES


2. UEDA, H., YASUDA, H., TAKABATAKE, Y.,

Japanese Circulation Journal Vol. 37, October 1973


5. WINER, N., CHOKSHI, D. S., YOON, M. S., & FREEDMAN, A. D.: Adrenergic receptor media-


14. UEDA, J.: Adenine nucleotide and renal function; Special reference with intrarenal distribution of blood flow. 45th General Meeting of Jap. Pharma-


Discussion:
Chairman: HISAKAZU YASUDA Tokyo Univ.

Dr. H. TAGAWA: I and Vander published the data indicating that infusion of cyclic AMP into renal artery at the doses of 0.5–1.0 mg/min caused inconsistent changes of renin secretion, while Winer found stimulation of renin release with the smaller doses. I think the dose of cyclic AMP in your experiment is too big to judge its physiological role in the mechanism of renin release. Have you tried the smaller doses of cyclic AMP?

Dr. K. YAMAMOTO: In our experiment, we have tried to infuse dibutyryl cyclic AMP at a dose of 0.3–0.5 mg/Kg/min into renal artery. One case in which 0.1 mg/Kg/min of dibutyryl cyclic AMP was infused, did not show marked increase in PRA.

Dr. H. TAGAWA: I doubt if cyclic AMP or dibutyryl cyclic AMP given in vivo could be incorporated into JG cells without structural changes. Therefore, we should be careful to conclude that cyclic AMP could stimulate renin release or be final pathway in the renin-secreting mechanism.

Dr. K. YAMAMOTO: I think it is necessary to know the metabolism of cyclic AMP. Therefore, we are just studying how cyclic AMP incorporate into kidney cells using 3H-cyclic AMP and 3H-butyryl cyclic AMP.

Dr. M. IIZUKA: 1) We have done the same in vivo experiment, intrarenal artery infusion of cyclic AMP and dibutryl cyclic AMP, with almost the same results. Two other groups in U.S.A have reported the same results. So, phenomenologically, the effects of cyclic AMP and dibutyryl cyclic AMP on overall renin release seem to have been almost established. 2) The β-receptor itself does exist in renal vasculature. As far as examined by hemodynamic effects, however, β-receptors connecting directly to the sympathetic nerve ending have not yet been confirmed in peripheral vascular beds. Thus, the theoretical pathway, renal nerve β-receptor-renin release can not be justified, until peripheral sympathetic nerve ending to β-receptor direct connection is clarified using other indices.

Dr. T. KISHIMOTO: There is no evidence that
sympathetic nerve endings connect to the \( \beta \)-receptors directly in the renal vascular bed. However, it should be remembered that there exist \( \beta \)-receptor which is stimulated by exogenous catecholamines (\( \beta \)-stimulator). Particularly, phe- 

Dr. H. YASUDA: In 1967, we have reported that intravenous infusion of isoproterenol stimulate increased release of renin which is inhibited or abolished by the administration of \( \beta \)-adrenergic blockade propranolol. In 1970, we found that intravertebral artery infusion of isoproterenol (about 1/7 amount of systemic infusion) caused increase in renin release. Recently, Dr. Reid et al. published a paper suggesting the existence of extrarenal mechanism of renin release during isoproterenol infusion. So, in your in vivo experiment, I think, the effect of systemic injection and intrarenal injection should be compared. Do you think that \( \beta \)-receptor which is stimulated by exogenous cyclic AMP and regulate renin release is located only in the kidney?

Dr. K. YAMAMOTO: I think that mechanism of stimulatory effect by isoproterenol is not completely exchangeable for the effect of cyclic AMP. The reasons are 1) isoproterenol does not stimulate renin release in our in vitro experiment 2) d-cyclic AMP has not only stimulatory effect but also inhibitory effect on renin release 3) the pattern and time course of renin release after administration are somewhat different in both drugs.

Dr. T. KISHIMOTO: In my opinion, mechanism of increase in renin release is a direct effect of isoproterenol on J. G. cells, because during intraarterial infusion 1) dose response curve is obtainable between isoproterenol and renin secretion 2) a small amount of propranolol inhibit the renin release even at a doses not inhibited the diuretic effect 3) the effect of systemic infusion is exaggerated more than intrarenal artery infusion possibly because of the hemodynamic effect or other factors.

Dr. H. SOKABE: When one discuss mechanism of renin release with such substances as adrenergic \( \beta \)-agonists, \( \beta \)-antagonists, cyclic AMP, dibutyryl cyclic AMP, one should consider first the pharmacological effects on renal vascular system (afferent or efferent arterioles), and renal sodium excretion besides their original actions on neuro-receptors or intracellular mechanism.

Dr. SARUTA: 1) I have reported yesterday that angiotensin suppressed the renin release from the kidney slices. Have you tried angiotensin in your in vitro experiment? 2) Previously, I have studied the effects of angiotensin and ACTH on adrenal cortex slices. In this experiment, it was found that angiotensin did not stimulate intracellular cyclic AMP which was different from ACTH. Do you have any ideas on the mechanism of inhibitory effect of angiotensin or renin secretion in related to cyclic AMP?

Dr. K. YAMAMOTO: We have not tried such experiment.