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Direct Action of Prostaglandins on Renin Release from Rat Renal Cortical Slices[1] (41095)

S. SUZUKI, R. FRANCO-SAENZ,[2] S. Y. TAN, AND P. J. MULROW

Department of Medicine, Medical College of Ohio, Toledo, Ohio 43699

Abstract. Various prostaglandins (PG's) and their precursors were superfused to rat renal cortical slices to evaluate their renin-stimulating actions. PGE_2, PGI_2, $PGF_{2\alpha}$, PGE_1, and PGD_2 stimulated renin release significantly. However, PGA_2 and 6-keto-$PGF_{1\alpha}$ did not stimulate renin release. The prostaglandin precursors, arachidonic acid and the endoperoxide analog, U-44069, also stimulated the release of renin. Since the renin-stimulating action of arachidonic acid was blocked by indomethacin, arachidonic acid-mediated renin release was caused by its conversion to the endoperoxides or to PG's. Our studies indicate that PG's may play an important role in the control of renin release. From our results, PGE_2 produced a significant increase in renin release at lower concentrations than any other PG's tested whereas PGI_2 caused the greatest percentage increase in renin release.

Recent reports suggest that PG's play an important role in renin release. Excessive synthesis of PG's in the kidney is probably involved in the abnormally high renin secretion in patients with Bartter's Syndrome (1). Indomethacin, an inhibitor of PG biosynthesis, diminishes renin release in patients with Bartter's Syndrome (2) and in normal subjects not only under basal conditions but also after stimulation by furosemide (3, 4).

In *in vivo* studies, PGE_2 (5), PGI_2 (6), PGD_2 (7), and PGA_1 (8) were reported to stimulate renin release in dogs and humans. However, because of the known hemodynamic and natriuretic effect of PG's, it is quite difficult to know whether this action is direct or indirect. *In vitro* studies in which two other important renin-controlling mechanisms such as the baroreceptor and macula densa mechanism are avoided enable us to provide a better model to investigate a direct action of PG's on renin release.

PGE_2, PGI_2, and $PGF_{2\alpha}$ were reported to stimulate renin release in rabbit and rat *in vitro* (9–12), however, it was also reported that PGE_2 did not stimulate renin release (11, 13) and $PGF_{2\alpha}$ (9, 13) inhibited renin release in the rabbit.

To clarify which PG's and PG precursors may stimulate the release of renin, a continuous superfusion system of rat renal cortical slices was used. This system enables us to study the same tissues as their own control and ensures the continuous delivery of PG's to the tissue.

Materials and Methods. Sprague–Dawley female rats weighing 200–250 g were used for the experiments. The rats were maintained on a regular Purina Chow diet and killed by decapitation. The kidneys were quickly removed and decapsulated. Cortical slices (approximately 0.5 mm thick) were prepared using a Stadie–Riggs microtome. Superficial cortical tissue was used and one slice was prepared from each side of each kidney. Sixty to eighty milligrams of renal cortical slices was used for superfusion system. The details of the superfusion method were recently reported by us (10). Renin concentration was measured by radioimmunoassay of angiotensin I by a modification of the method of Haber *et al.* (14). Renin substrate was obtained from rat plasma which was collected 24 hr after bilateral nephrectomy. Fifty microliters of superfusion samples was used for angiotensin I generation. Angiotensin I generation medium was composed of 75 μl of renin substrate, 90 μl of Tris–acetate–lysozyme Buffer, 25 μl of 4% EDTA, 5 μl dimercaprol and 5 μl of 8-hydroxyquinoline and was incubated for 3 hr at 37°. Fifty mi-

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[2] To whom reprints should be addressed.

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croliter aliquots were taken for radioimmunoassay. The interassay coefficient of variation using superfusate samples was 11.5% (N = 10) and intraassay coefficient of variation was 10.5% (N = 10). The recovery of angiotensin I added to superfusate was 85 ± 2% (N = 10). The results of the experiments are expressed as the percentage increase over the baseline renin release after each stimulation.

PG's and endoperoxide analog (U-44069) were obtained from the Upjohn Company (Kalamazoo, Mich.) and indomethacin, and arachidonic acid were obtained from Sigma Chemical Company (St. Louis, Mo.). PG's, indomethacin, and arachidonic acid were first dissolved in ethanol at the concentration of 1 mg/ml and were evaporated by nitrogen gas just before preparing the solution. The pH of these solutions was adjusted to 7.4 except the PGI_2 solution which was adjusted to pH 9.0. The buffer of the control period of the PGI_2 experiment was also adjusted to pH 9.0. The results of the experiments are expressed as the percentage increase over the baseline renin release after each stimulation. Statistical analysis was performed by the paired t test with log transformation. Significance was defined as a P value of less than 0.05.

Results. Figure 1 shows that PGE_2, PGI_2, and $PGF_{2\alpha}$ stimulated renin release significantly. Although PGE_2 was the most effective stimulator of renin release, PGI_2 was the most powerful stimulator of renin release causing a 381.6 ± 180.7% increase at concentrations of 10^{-4} M. $PGF_{2\alpha}$ also caused a significant increase of renin release but required a higher concentration (6 × 10^{-5} M or higher).

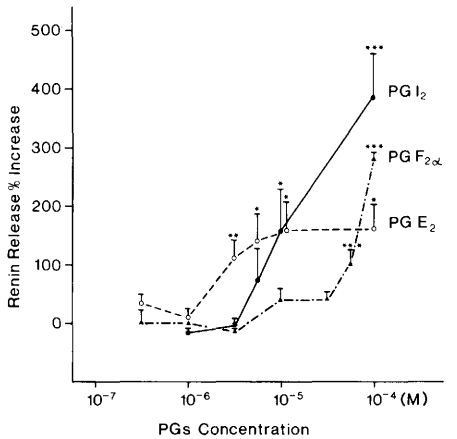

FIG. 1. Effects of PGE_2, PGI_2, and $PGF_{2\alpha}$ on renin release. Values represent the percentage increase over control. Each point represents the mean ± SE of 4 to 10 experiments. *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.005$.

Figure 2 shows that PGD_2 and PGE_1 also stimulated renin release at a concentration of 10^{-5} M or higher but the effect was weaker than that of PGI_2 and PGE_2. However, at a concentration of 10^{-5} M, PGD_2 and PGE_1 were more potent than $PGF_{2\alpha}$. PGA_2 and 6-keto $PGF_{1\alpha}$ did not stimulate renin release at concentration from 10^{-6} to 10^{-4} M.

The effects of arachidonic acid and endoperoxide analogue (U-44069) on renin release are shown in Fig. 3. Arachidonic acid, 10^{-5} M, increased renin release by 141.1 ± 49.2%, however, no further increase of renin release was observed with a higher concentration of arachidonic acid (10^{-4} M). The endoperoxide analog (U-44069) (15 S) hydroxy-9α, 11α-(epoxymethano)prosta-5Z,13E-dienoic acid, 10^{-5} M, increased renin release by 125.0 ± 23.8% with a further increase to 199.9 ± 64.6% at 10^{-4} M.

Figure 4 shows the effects of indomethacin on renin release stimulated by arachidonic acid and the endoperoxide analogue. Arachidonic acid (10^{-5} M) stimulation of renin release was completely abolished by indomethacin (10^{-4} M), whereas, endoperoxide analog (10^{-5} M)-stimulated renin release was not blocked by indomethacin (10^{-4} M).

Discussion. In 1968, Vander (15) reported that PGE_1 and PGE_2 did not stimulate renin release in anesthetized antidiuretic dogs. They used relatively small doses of prostaglandins which did not change the blood pressure and heart rate. On the other hand, Werning *et al.* (16) and several other investigators (5–8) have shown that infusion of PGE_1, PGE_2, PGI_2, PGD_2, and PGA_2 stimulated renin release in the dog, rabbit, and human. However, due to the hemodynamic and natriuretic effect of

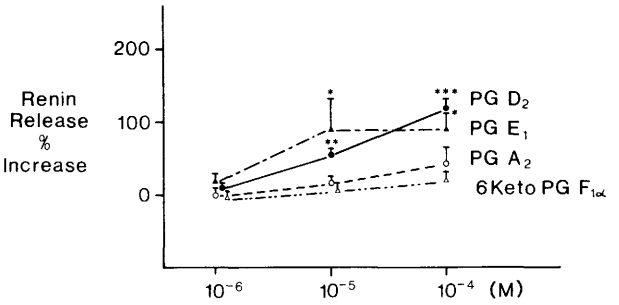

FIG. 2. Effects of PGD_2, PGE_1, PGA_2 and 6-keto $PGF_{1\alpha}$ on renin release. Each point represent the mean ± SE of 4 experiments. PGD_2 10^{-5} to 10^{-4} *M*, PGE_1 10^{-5} to 10^{-4} *M* stimulated renin release significantly. PGA_2 and 6-keto $PGF_{1\alpha}$ did not stimulate renin release. *,$P < 0.05$; **,$P < 0.01$; ***,$P < 0.005$.

PG's it is difficult to conclude that PG's have a direct effect on renin release. Dew *et al.* (9) using an *in vitro* system of rabbit renal cortical cell suspension reported preliminary evidence that PGE_2 stimulated renin release and that $PGF_{2\alpha}$ inhibited the release of renin. However, Corsini *et al.* (17) failed to show stimulation by PGE_1 in rat renal cortical slice and Weber *et al.* (13) reported that renin release was stimulated by arachidonic acid and endoperoxide analogs (U-44069) but failed to show stimulation by PGE_2. $PGF_{2\alpha}$ inhibited renin release in their rabbit renal cortical slice system. Whorton *et al.* (11) reported that PGI_2 stimulated renin release but PGE_2 did not. Our experiment using a continuous superfusion system which prevents the degradation of PG's demonstrated that a number of PG's (PGE_1, PGE_2, PGI_2, PGD_2, and $PGF_{2\alpha}$) stimulate renin release from rat renal cortical slices. In our system PGE_2 appears to be most effective and PGI_2 is the most potent stimulator of renin release. However, due to the known instability of PGI_2, it is difficult to make this comparison. It was in-

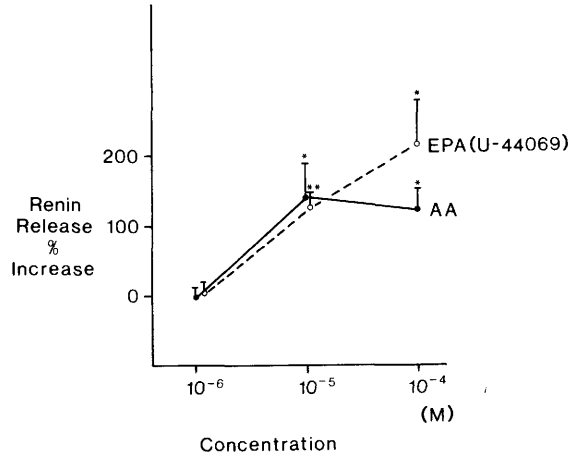

FIG. 3. Effects of arachidonic acid and endoperoxide analogue (EPA, U-44069) on renin release. Values represent the percentage increase over control. Each point represents the mean ± SE of four experiments. Arachidonic acid (AA) 10^{-5} to 10^{-4} *M* and endoperoxide analog 10^{-5} to 10^{-4} *M* stimulated renin release. *,$P < 0.05$, **,$P < 0.01$.

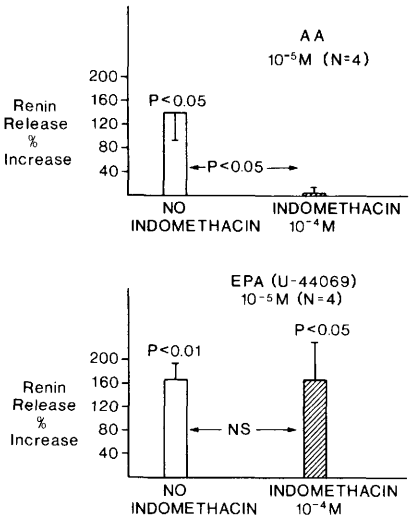

FIG. 4. Effects of indomethacin on renin release stimulated by arachidonic acid and endoperoxide analog (EPA). Each point represents the mean ± SE of four experiments. Indomethacin, 10^{-4} *M*, completely abolished the arachiodonic acid stimulated renin release, however, did not inhibit the endoperoxide analog-stimulated renin release.

teresting to find that $PGF_{2\alpha}$ also stimulated the release of renin but at a higher concentration than that of other PG's. Although it was initially reported that $PGF_{2\alpha}$ inhibited renin release and PGE_2 did not stimulate renin release *in vitro*, a recent preliminary report by Whorton *et al.* (18) and Lin *et al.* (19) agreed with our finding that $PGF_{2\alpha}$ and also PGE_2 stimulates renin release *in vitro*. The effect of PGD_2 on renin release has been the subject of controversy. Seymour *et al.* (20) reported that PGD_2 (10^{-8} to 10^{-7} g/kg/min) stimulated renin release from filtering and nonfiltering kidney. On the other hand, Gerber *et al.* (6) reported that infusion of PGD_2 (10^{-8} to 10^{-7} g/kg/min) into the renal artery of anesthetized dogs did not stimulate renin secretion. Recently, Whorton *et al.* (18) reported that PGD_2 was completely inactive on renin release from rabbit renal cortical slices. Our result demonstrates that PGD_2 causes mild but significant increase in renin release *in vitro*. Our experiments also agree with the reports (21) that arachidonic acid-stimulated renin release is blocked by the cyclooxygenase inhibitor, indomethacin. The effect of arachidonic acid on renin release is due to its conversion to either endoperoxide or primary PG's. Whether the endoperoxide analog (U-44069) has a direct effect on renin release or its effect is due to its conversion to primary PG's is not clarified by our experiments. In conclusion, our results indicate that a number of PG's cause direct stimulation of renin release from rat renal cortical slices. PGE_2 produced a significant increase in renin release at lower concentrations than any other PG's tested whereas PGI_2 caused the greatest percentage increase in renin release.

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