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Prostaglandins and Renin Release from Submaxillary Glands *in Vitro* (41589)

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Abstract. The present studies were undertaken to investigate the effect of prostaglandins (PGs) on renin release from the submaxillary glands of mice. Pooled mouse submaxillary gland slices were incubated in Krebs–Henseleit buffer solution following a preincubation period, and renin release was measured by a radioimmunoassay for the direct measurement of submaxillary gland renin. Arachidonic acid (AA) significantly stimulated renin release at 10, 20, and 30 min of incubation. These increases of renin release were abolished by the presence of indomethacin. The synthetic prostaglandin endoperoxide analogue (EPA) strongly stimulated renin release at 10, 20, and 30 min of incubation. However, at a higher concentration the stimulating effect of EPA virtually disappeared. PGI_2 caused the highest increase of renin release at 10 and 20 min of incubation. At higher concentrations the effect of PGI_2 on renin release was drastically reduced, although it was still statistically significant. PGE_2 and $PGF_{2\alpha}$ also exerted a significant increase in renin release; however, the extent of this effect was much less than that of EPA and PGI_2. Other prostaglandins such as PGE_1, PGA_2, PGD_2, $PGF_{1\alpha}$, and 6-keto-$PGF_{1\alpha}$ were found to have no significant effect on renin release. These results suggest that the prostaglandin system directly affects renin release from submaxillary gland independent of systemic hemodynamic and neurogenic influences.

Renal renin is synthesized and stored in the juxtaglomerular apparatus and its physiological or pathological importance has been emphasized. Organs such as uterus, submaxillary glands, and others have also been shown to contain renin. The presence of large amounts of renin in the submaxillary gland has been demonstrated by several investigators (1–3). In most of the studies, renin has been measured indirectly as enzymatic activity (angiotensin I generated from renin substrate), which may not always represent actual renin levels. This can be of importance since high-molecular weight renin has considerably less enzymatic activity than low-molecular weight renin (4–6). We have developed a radioimmunoassay for the direct measurement of renin (7) in tissues and biological fluids. This direct measurement of renin can provide a more accurate assessment of renin levels. Using this procedure we have shown that the sympathetic nervous system plays an important role in mediating renin release from submaxillary gland and kidney (8, 9). In these studies it was found that the beta-adrenergic agonist, isoproterenol, stimulates renin release from the kidney. In contrast, the alpha-adrenergic agonist phenylephrine stimulates renin release from the submaxillary gland in mice. In further studies we also found that, by way of different mechanisms, both isoproterenol and phenylephrine increase big and small plasma renin simultaneously although the increase of small renin was significantly greater than that of big renin (5). Recently prostaglandins have been implicated in the release of renal renin (10). Prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, PGI_2, and PG endoperoxide analogue (EPA) have been shown to stimulate renin release from mouse renal cortex *in vitro* (11). However, it is not known whether prostaglandins have any effect on the release of renin from submaxillary glands. The present study was performed to examine the effect of prostaglandins on the release of renin from mouse submaxillary glands *in vitro* using the radioimmunoassay procedure (7) for the direct measurement of renin.

Materials and Methods. *Materials.* Arachidonic acid (AA), isoproterenol HCl, phenylephrine HCl, and indomethacin were purchased from Sigma Chemical Co. (St. Louis, Mo.). All prostaglandins and 15 S-hydroxy-9α,11α-(epoxymethano)prosta-5Z,13E-dienoic acid (EPA) were generous gifts from the Upjohn Co. (Kalamazoo, Mich.). All chemicals were dissolved in absolute ethanol and stored at −70°C except PGI_2 sodium salt, 6-

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keto $PGF_{1\alpha}$, phenylephrine HCl, and isoproterenol HCl, which were prepared in Krebs–Henseleit (K-H) buffer solution (pH 7.4) immediately before use.

Experimental procedures. Adult male Swiss albino mice, weighing 30–40 g, were used in all experiments. The mice were anesthetized with pentobarbital (60 mg/kg), and their submaxillary glands were removed and immediately placed in K-H buffer solution (pH 7.4, 4°C). The glands were washed twice in K-H buffer and cut into small slices (six to eight per gland), with a razor blade. Two-hundred milligrams of the slices were placed in each plastic tube (8.2 × 1.6 cm) containing 2 ml of K-H buffer solution. The same submaxillary glands were used in the control and the treated samples and all samples were run in triplicate. The slices were then preincubated at 37°C for 30 min with gentle shaking under an atmosphere of 95% O_2 and 5% CO_2. At the end of preincubation, the medium was quickly aspirated and replaced with 2 ml of fresh buffer solution. Ten microliters of indomethacin, AA, EPA, PGI_2, PGE_2, $PGF_{2\alpha}$, PGE_1, PGA_2, PGD_2, $PGF_{1\alpha}$, 6-keto-$PGF_{1\alpha}$, phenylephrine, and isoproterenol at different concentrations (1.5×10^{-10} to 1.5×10^{-4}) were then added to the slices, immediately followed by incubation for 30 to 60 min at previously described preincubation conditions. For the control experiments, 10 μl of absolute ethanol or buffer were added. During the incubation period 25-μl samples were drawn at 10, 20, 30, and 60 min intervals and diluted to 2 ml with 50 m*M* phosphate buffer solution containing 0.5% bovine serum albumin, pH 7.4, and then frozen at −20°C until assayed for renin.

Direct measurement of renin. A radioimmunoassay modified slightly from that previously described (7) was used to measure renin. In brief, mouse submaxillary renin was purified according to Cohen *et al.* (12). For the production of renin antibody, one milligram of submaxillary renin was mixed with Freund's complete adjuvant and then injected into rabbits once a week for 4 weeks. One week after the fourth injection, plasma with high anti-renin titer was obtained and used in the radioimmunoassay procedure. Radioiodinated renin was prepared as described in a previous report (7). For the measurement of renin, the unknown sample in each tube (7.5 × 1.2 cm) was mixed with labeled renin (3000–6000 cpm/100 μl), renin antiserum (1:300,000 dilution), 50 m*M* sodium phosphate buffer, pH 7.4, and the total volume was adjusted to 1 ml with 0.5% bovine serum albumin. The sample was then incubated for 72 hr at 4°C. At the end of this incubation, 100 μl of normal rabbit serum (1:20 dilution) and 100 μl of goat anti-rabbit Ig G serum (1:4 dilution, from Research Products International Corp.) were added to the samples, which were then reincubated for another 24 hr at 4°C. After this final incubation the samples were centrifuged at 3000 rpm for 30 min. The radioactivity contained in both the supernatant and the precipitate was measured by a Packard Gamma Scintillation Spectrometer (Model 3001). The AA, indomethacin, prostaglandins, and EPA at 1.5×10^{-6} *M* did not interfere with the binding of labeled renin to renin antibody.

Statistical analysis was done using analysis of variance. The data were presented as mean ± SD.

Results. *Effects of adrenergic agonists on submaxillary renin release.* The alpha-adrenergic agonist phenylephrine stimulated submaxillary renin release as shown in Fig. 1.

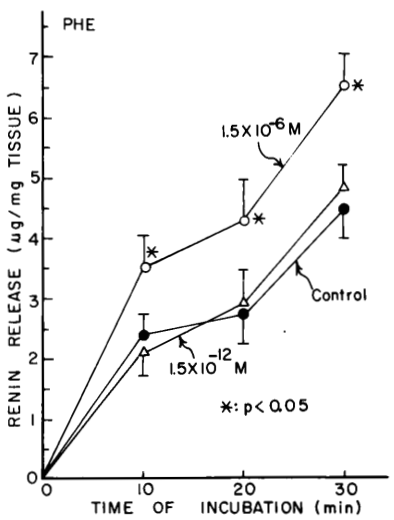

FIG. 1. Time course of renin release from submaxillary gland slices following incubation with or without phenylephrine (PHE) at concentrations of 1.5×10^{-12} *M* and 1.5×10^{-6} *M*. Each point represents mean ± SD from 10–12 determinations.

Phenylephrine at 1.5×10^{-12} *M* did not have any significant effect on renin release. At 1.5×10^{-6} *M* phenylephrine significantly increased renin release over control ($P < 0.05$). The beta-adrenergic agonist isoproterenol at 1.5×10^{-6} *M*, 1.5×10^{-9} *M*, or 1.5×10^{-12} *M* caused a slight inhibition of renin release, however this was not statistically significant.

Effects of AA and indomethacin on submaxillary renin release. The time course of renin release when stimulated by AA is shown in Fig. 2. At low concentration (1.5×10^{-12} *M*) AA did not exert any significant effect. At higher concentration (1.5×10^{-7} *M*) AA significantly stimulated renin release ($P < 0.05$) at 10, 20, and 30 min of incubation. These increases were abolished by the simultaneous treatment with indomethacin (1.5×10^{-6} *M*). Indomethacin alone (1.5×10^{-6} *M*) seemed to decrease the basal release of submaxillary renin, however, this was not statistically significant.

Effects of EPA on submaxillary renin release. EPA, a synthetic prostaglandin endoperoxide, was a strong stimulator of renin release as shown in Fig. 3. At 10 min of incubation EPA (1.5×10^{-12} *M*) significantly increased renin release (109% over control, $P < 0.01$). With longer incubation at this concentration, EPA also caused a significant renin release ($P < 0.05$). At higher concentration (1.5×10^{-9} *M*) the increase of renin release virtually disappeared. This may suggest that

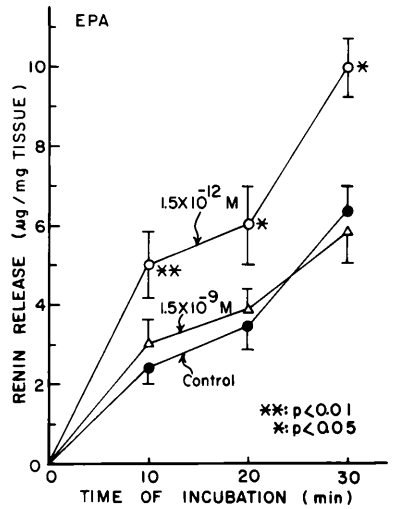

FIG. 3. Time course of renin release from submaxillary gland slices following incubation with or without EPA (1.5×10^{-12} *M* and 1.5×10^{-9} *M*). Each point represents mean ± SD from 10–12 determinations.

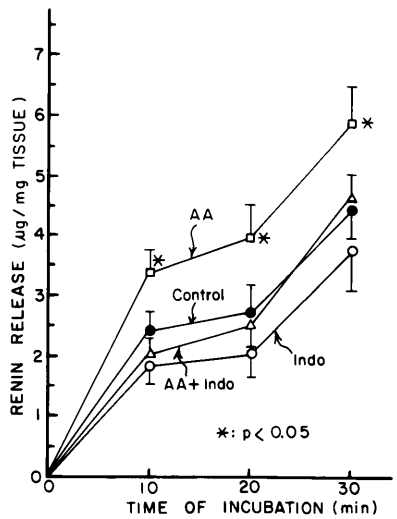

FIG. 2. Time course of renin release from submaxillary gland slices following incubation with either arachidonic acid (AA 1.5×10^{-7} *M*), or indomethacin (Indo 1.5×10^{-6} *M*), or both. The control experiments are those incubated with buffer only. Each point represents mean ± SD from 10–12 determinations.

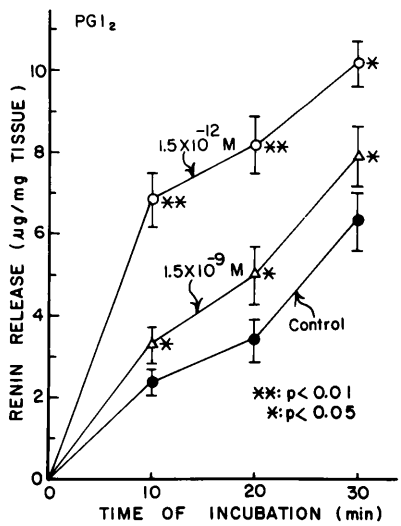

FIG. 4. Time course of renin release from submaxillary gland slices following incubation with or without PGI_2 (1.5×10^{-12} *M* and 1.5×10^{-9} *M*). Each point represents mean ± SD from 10–12 determinations.

EPA exerts a biphasic effect on the release of submaxillary renin.

Effects of PGI_2 on submaxillary renin release. PGI_2 was also found to be a strong stimulant for the release of submaxillary renin as shown in Fig. 4. At 10 min of incubation PGI_2 (1.5×10^{-12} *M*) almost tripled the release of renin (192% over control, $P < 0.01$). A significant increase of renin release was also found at 20 and 30 min of incubation with this concentration of PGI_2. At higher concentration (1.5×10^{-9} *M*) the increase of renin release declined. At 10, 20, and 30 min, renin release was stimulated from 2.37 ± 0.27 to 3.33 ± 0.42, from 3.41 ± 0.49 to 4.94 ± 0.76, and from 6.34 ± 0.83 to 7.91 ± 0.77 μg/mg, respectively. This suggests that PGI_2 as well affects renin release in a biphasic pattern.

Effects of PGE_2 on submaxillary renin release. At 10 min of incubation PGE_2 (1.5×10^{-12} *M*) caused an increase of renin release which represents 67% over control ($P < 0.05$) as shown in Fig. 5. With further incubation with PGE_2 at 20, 30, and 60 min the increase of renin release was also statistically significant ($P < 0.05$).

Effects of $PGF_{2\alpha}$ on submaxillary renin release. At 10 min of incubation $PGF_{2\alpha}$ (1.5×10^{-12} *M*) also stimulated renin release 50%

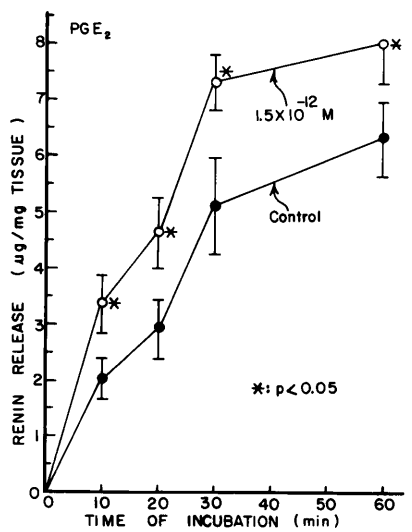

FIG. 5. Time course of renin release from submaxillary gland slices following incubation with or without PGE_2 (1.5×10^{-12} *M*). Each point represents mean ± SD from 10–12 determinations.

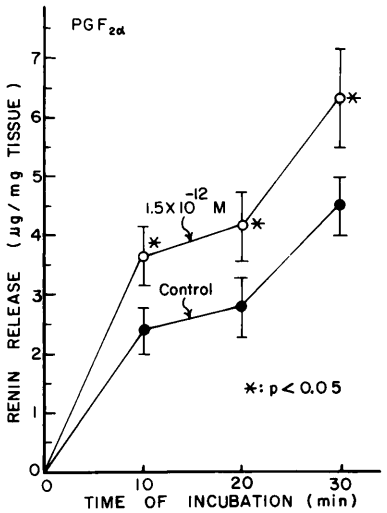

FIG. 6. Time course of renin release from submaxillary gland slices following incubation with or without $PGF_{2\alpha}$ (1.5×10^{-12} *M*). Each point represents mean ± SD from 10–12 determinations.

over control ($P < 0.05$) as shown in Fig. 6. At 20 and 30 min of incubation with $PGF_{2\alpha}$ the increase of renin release was also statistically significant ($P < 0.05$).

Effects of other prostaglandins on submaxillary renin release. The effects of PGE_1, PGA_2, PGD_2, $PGF_{1\alpha}$, and 6-keto-$PGF_{1\alpha}$ on renin release from the submaxillary gland have also been investigated. At concentrations of 1.5×10^{-12} *M*, 1.5×10^{-9} *M*, and 1.5×10^{-6} *M* they were found to have no significant effect throughout the incubation periods.

Discussion. In a previous study we reported the effect of prostaglandins on renin release from the kidneys (8). In this study, we describe the effect of prostaglandins on renin release from mouse submaxillary glands *in vitro*. For the measurement of renin a radioimmunoassay procedure for the direct measurement of renin (7) was used. This procedure provides more accurate assessment of total renin release since it can measure renins of different molecular weights in contrast to the plasma renin activity procedure which measures only enzymatically active renin.

Among the various agents studied, we found that AA, PGE_2, $PGF_{2\alpha}$, EPA, PGI_2, and phenylephrine increased the release of submaxillary renin, but PGA_2, PGD_2, PGE_1, $PGF_{1\alpha}$, 6-keto-$PGF_{1\alpha}$, and isoproterenol had

no effect. Phenylephrine which specifically stimulates alpha-adrenergic receptors caused release of renin from submaxillary gland, whereas the beta-adrenergic agonist, isoproterenol, did not. These findings, that phenylephrine but not isoproterenol stimulated submaxillary renin release *in vitro*, are consistent with our previous studies (8, 9) which showed that submaxillary renin release is mediated through α- but not β-adrenergic receptors.

AA stimulated submaxillary renin release; its effect was abolished by indomethacin. This suggests that AA may convert to prostaglandins or other metabolites to exert its effect since this event can be prevented by indomethacin. However, the possibility that the blockade of submaxillary renin release as stimulated by AA is unrelated to inhibition of prostaglandin cyclooxygenase, cannot be excluded.

EPA, a stable analogue of PGH_2, was used to simulate the effect of PGH_2 on submaxillary renin release. EPA and PGI_2 (1.5×10^{-12} M) were found to be very strong stimulators. The response of submaxillary renin release to EPA or PGI_2 was rapid and dramatic. However, at higher concentration (1.5×10^{-9} M) the amount of submaxillary renin release was decreased in the case of PGI_2 and returned to control level in the case of EPA. This suggests that EPA and PGI_2 may exert a biphasic effect on the release of submaxillary renin, the same phenomenon exhibited in their effects on kidney renin (11). It is possible that EPA may be converted to PGI_2 to exert its stimulant effect. PGI_2, on the other hand, is apparently the major active prostaglandin for stimulating submaxillary renin release, since its metabolite, 6-keto-$PGF_{1\alpha}$, did not show release-stimulating activity. The exact mechanism by which PGI_2 and EPA exert their effect is still unknown. Whether they act directly, or through the interaction with certain activating enzyme systems such as adenylate cyclase, remains to be investigated.

$PGF_{2\alpha}$ and PGE_2 at 1.5×10^{-12} M were found to have significant effects on mouse submaxillary renin release, with similar potency, although their effects were much less than those of PGI_2 and EPA. It may be possible that PGE_2 was converted in one way or another by 9-keto reductase (13) to exert its stimulant effect, if the enzyme is present in the submaxillary gland. Whether PGE_2 and $PGF_{2\alpha}$ themselves or their actual metabolites caused the effect still needs to be clarified.

Our study also showed that at 1.5×10^{-12} M, 1.5×10^{-9} M, and 1.5×10^{6} M PGA_2, PGD_2, PGE_1, $PGF_{1\alpha}$, and 6-keto-$PGF_{1\alpha}$ had no significant effect on renin release from mouse submaxillary gland slices. Therefore, the pattern of prostaglandin effects on renin release from mouse submaxillary gland is generally similar to that of release from mouse renal cortex, although the strength of stimulation by prostaglandins may not be comparable in both organ systems.

In this study, we found that PGI_2 and EPA are very strong stimulators of submaxillary renin release, followed by PGE_2, $PGF_{2\alpha}$, AA, and phenylephrine. Our results suggest that the prostaglandin system affects renin release from the submaxillary gland independent of systemic hemodynamic and neurogenic influences. It is noteworthy that both the PG system and the α-adrenergic system can independently stimulate submaxillary renin release. However, the degree of contribution by each individual system to submaxillary renin release, is very difficult to assess and remains to be investigated.

In addition, the physiologic role of submaxillary renin remains unknown. It appears that submaxillary glands do not normally have significant effects on plasma renin concentrations (5, 8, 14). However, it is possible, that during alarm or stress situations submaxillary renin may be released into the blood stream and together with renal renin increase arterial blood pressure. It is also possible that submaxillary renin may be involved in the digestive process since it is largely released into the saliva (7).

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