SUMMARY The mechanism by which renal prostaglandins stimulate renin secretion in vivo is unknown. In this in vitro study we measured the effects of activation of the prostaglandin (PG) system on renin release from slices of rabbit renal cortex. The PG precursor arachidonic acid (C20:4), a natural PG endoperoxide (PGG2), two stable synthetic PG endoperoxide analogues (EPA I and II), PGE2, PGF2α, and two different PG synthesis inhibitors (indomethacin and 5,8,11,14-eicosatetraynoic acid (ETA)) were used to evaluate the possibility of a direct action of the cortical PG system on renin secretion. Renin release increased significantly with time after addition of C20:4, PGG2, EPA I, and EPA II to the incubation medium. Stimulation of renin release was dose-related for C20:4 in concentrations of 0.6 to 4.5 $\times 10^{-5}$ M, for EPA I in concentrations of 0.7 to 2.8 $\times 10^{-5}$ M, and for EPA II in concentrations of 1.4 to 14.0 $\times 10^{-5}$ M. Indomethacin (10$^{-4}$ M) and ETA (10$^{-4}$ M) had no effect on renin release, whereas PGF2α (10$^{-4}$ to 10$^{-6}$ M) decreased renin release in a dose-dependent manner. These data raise the possibility of a direct action of the renal cortical PG system on renin secretion. The results further indicate that stimulation of renin release by C20:4 may depend more specifically on the action of PG endoperoxides than on the primary prostaglandins.

THE FUNCTION of the kidney and its influence on sodium and water homeostasis and blood pressure are determined by a variety of integrated neurogenic and hormonal systems. These include the renin-aldosterone, renin-antidiuretic hormone (ADH), renin-catecholamine, and macula densa-glomerular filtration rate (GFR) interrelationships. Stimulation of the renin-angiotensin system results in the production of angiotensin II (A II); this can occur both locally within the kidney and at extrarenal sites. The action of the renin-angiotensin system on kidney function might be determined by the amount of A II produced intrarenally and in the systemic circulation. This in turn would depend on the degree of renin release and activation within and the extent of renin secretion from the kidney. The present study addresses itself to a new aspect in the control of renin secretion, namely, an interaction with the renal prostaglandin (PG) system.

Stimulation of renal PG biosynthesis by the administration of arachidonic acid to rabbits and dogs increases renal blood flow preferentially in the juxtamedullary cortex and enhances urinary volume and sodium output. Infusion of arachidonic acid to rabbits and rats also increases plasma renin activity. On the other hand, inhibition of intrarenal PG biosynthesis by nonsteroid anti-inflammatory drugs attenuates renal blood flow, GFR, urinary volume, and sodium output. These drugs also decrease plasma renin activity. The effect of prostaglandins on the release of renin could be direct or an indirect one secondary to PG-modulated changes in renal function. The localization of renin predominantly in the kidney cortex and the recent demonstration of PG biosynthesis also in this region led us to test the hypothesis that there might be a direct interaction between these two systems. The PG endoperoxides, earlier postulated to be intermediates in the biosynthesis of the prostaglandins, recently have been isolated. Studies on the biological properties of these compounds have indicated that they are extremely potent in several biological systems. The endoperoxides from arachidonic acid, prostaglandin G2 (PGG2) (Fig. 1), and prostaglandin H2 (PGH2) are
The instability of PGG2, PGH2, and thromboxane A2 has been a limitation in studies of their biological properties. However, recently synthetic stable endoperoxide analogues (EPA) have become available.

The structures of two of these analogues, EPA I and II, are shown in Figure 1. In the present study we have examined the effects of arachidonic acid, PGG2, EPA I and EPA II, PGE2, and PGF2α on the release of renin from slices of rabbit renal cortex.

Methods

MATERIALS

Arachidonic acid (C20:4) was stored as the methyl ester and hydrolyzed just before an experiment. The hydrolyzed acid was isolated by silicic acid chromatography and dissolved in alcohol-buffer (1:10) before addition to the incubation medium to give final concentrations of 0.6 x 10^{-8} to 1.5 x 10^{-4} M.

PGG2 was isolated as described previously. It was dissolved and stored in dry acetone at -20°C. To each sample was added 10 μl of EPA I or II, respectively, to give final concentrations of 0.6 x 10^{-6} to 2.24 x 10^{-5} M.

EPA I and II were dissolved and stored in dry acetone or absolute alcohol in -20°C. To each sample were added 10 μl of EPA I or II, respectively, to give final concentrations of 0.6 x 10^{-8} to 2.24 x 10^{-5} M.

PGG2 and PGE2 were dissolved in alcohol-buffer (1:10) just before an experiment and added to the incubation medium to give final concentrations of 1.0 x 10^{-4} to 4.5 x 10^{-6} M.

Indomethacin and 5,8,11,14-eicosatetraynoic acid (ETA) were dissolved in alcohol-buffer (1:10). For pretreatment by intravenous injection, indomethacin or ETA (5 mg/ml) were given (5 mg/kg of body weight) 1 hour and 10 minutes before taking out the kidneys. For the experiments in vitro these inhibitors were dissolved in alcohol-buffer (1:10) and added to the incubation medium to make a final concentration of 10^{-4} M.

KIDNEY SLICES

Crossbred rabbits, weighing between 2 and 3 kg, were maintained on a standard diet. Kidney slices were prepared and incubated as follows: The left kidney was removed quickly under pentobarbital anesthesia (40 mg/kg, iv), decapsulated, and placed in an ice-cold sodium bicarbonate buffer. Fifty to sixty slices (10-20 mg, wet weight, and 0.3-0.5 mm in thickness) were prepared from a cortical kidney segment using a razor blade. The segment was obtained by cutting out a slice 2-3 mm wide and 2-2.5 mm thick along the widest circumference of the kidney. Five to seven slices were combined in a plastic tube containing 2 ml of Krebs-Henseleit buffer adjusted to pH 7.4 and containing 0.154 M NaCl (100 parts), 0.154 M KCl (4 parts), 0.154 M NaHCO3 (21 parts), 0.154 M KH2PO4 (1 part), 0.11 M CaCl2 (3 parts), 0.154 M MgSO4 (1 part), and glucose (200 mg/100 ml). After 8-10 tubes were prepared, the buffer was quickly aspirated and fresh medium was added (2 ml to each tube) before starting the incubation period.

INCUBATION PROCEDURE FOR SLICES

Incubations were performed at 37°C with shaking under an atmosphere of 95% O2 and 5% CO2. After the preincubation period, which varied between 15 and 45 minutes for different experiments, and during the experimental periods 50-μl samples were removed for renin analysis. The samples were briefly centrifuged at +4°C and the supernatant fluid was quickly frozen and stored at -20°C until analyzed.

EXPERIMENTAL SERIES

Time Course of Effect of Arachidonic Acid

Renin determinations were performed after a 45-minute preincubation period and 30 and 60 minutes after addition of C20:4.

Dose-Response Curve for Arachidonic Acid

The effect of arachidonic acid (0.6 x 10^{-8} to 1.5 x 10^{-4} M) was studied after a preincubation period of 30 minutes with an experimental period of 30 minutes. Dose-response curves for C20:4 also were performed after pretreatment in vivo of rabbits either with indomethacin or with ETA. In the experiments with pretreated rabbits, indomethacin or ETA also was added to the incubation medium during the experiment.

Dose-Response Curve for PGG2

Because of the instability of this compound it was added repeatedly every 5 minutes to achieve concentrations of 1.0 x 10^{-8} to 4.5 x 10^{-4} M after a preincubation period of 15 minutes. The samples for renin analysis were removed after the preincubation period and after an experimental period of 15 minutes.

Time Courses for EPA I and EPA II

Samples were analyzed for renin after a 15- to 20-minute preincubation period and 5, 10, and 25 minutes...
after addition of EPA I (2.8 × 10^{-8} M) or EPA II (14 × 10^{-8} M), respectively.

**Dose-Response Curves for EPA I and EPA II**

Samples were analyzed for renin after a preincubation period of 15 minutes and an experimental period of 15 minutes with EPA I (0.7 × 10^{-8} to 2.24 × 10^{-6} M) or EPA II (1.4 × 10^{-4} M to 1.4 × 10^{-5} M). A dose-response curve was also obtained for EPA I after pretreatment of the rabbits with indomethacin.

**Dose-Response Curves for PGE_{2} and PGF_{2α}**

The effects of PGE_{2} and PGF_{2α} (10^{-11} to 10^{-6} M) were studied after a 15-minute preincubation period and with an experimental period of 15 minutes.

**DETERMINATION OF RENIN RELEASE**

The renin levels were measured by adding 10 μl of the samples to 40 μl of a 200 mM Na phosphate buffer, pH 7.0, containing purified rabbit renin substrate in excess (4.500 ng/ml, expressed as angiotensin I). The sodium salt of ethylenediaminetetraacetic acid (EDTA) (final concentration, 3.5 × 10^{-3} M) and diisofluorophosphate (DFP) (final concentration, 1.3 × 10^{-3} M) were added to inhibit angiotensinase and converting enzyme activity in the renin sample. The rabbit renin substrate was prepared as described earlier and contained 400 ng/mg protein (expressed as angiotensin I). The samples were incubated for 60 minutes at 37°C. After termination of the reaction by boiling, the incubate was diluted 1:9 by adding 400 μl of ice-cold saline. After centrifugation samples of 10-50 μl were used for the radioimmunoassay, which is based on the method of Haber et al. and has been described in detail previously. Control experiments were performed to measure the influence of arachidonic acid and the primary prostaglandins on the renin-renin substrate reaction in our incubation system. C20:4, PGE_{2}, and PGF_{2α} had no effects on the angiotensin formation rate in vitro after the addition of each of these substances separately in concentrations of 10^{-7} to 10^{-6} M to a standardized renin-renin substrate mixture.

**CALCULATIONS**

In each incubation study the renin value obtained after the preincubation period served as control for the consecutive experimental period(s). The calculation of renin values was performed in two ways. In the time course experiments the results are expressed as percent change from the preincubation value. In the dose-response experiments the results are expressed as percent change from control (no addition) at the same time in the experimental period.

Absolute values for renin release were calculated as the amounts of angiotensin I formed on incubation of the renin sample with renin substrate. The values are expressed as picograms of angiotensin I per minute per milligram of tissue (wet weight). Statistical analysis was performed using Student's t-test. For all figures * = P < 0.05; ** = P < 0.025; *** = P < 0.01; **** = P < 0.001; † = not significant. Values are presented as mean ± SE.
somewhat more effective in comparison to indomethacin. At higher C20:4 concentrations (1.5 × 10⁻⁴ M) the inhibitory action of indomethacin was abolished and that of ETA was diminished.

**EFFECTS OF PG ENDOPEROXIDES**

Because of the instability of PGG₂ in aqueous solutions it was not possible to make accurate studies of the time- and concentration-dependence of the effect on renin release. At concentrations of 1 × 10⁻⁸ M and 2 × 10⁻⁶ M, PGG₂ caused a 51 ± 22% and 54 ± 15% increase (n = 6 for each concentration) in renin release (P < 0.05). However, at 4.5 × 10⁻⁶ M the increase was only 30 ± 12% (n = 6).

The time-dependencies for the action of EPA I and II are shown in Figure 4. After addition of 2.8 × 10⁻⁶ M EPA I (upper panel), renin release increased linearly up to 10 minutes. After that time the rate of release paralleled control. After addition of 1.4 × 10⁻⁵ M EPA II (lower panel), renin release was stimulated linearly during the observation period of 25 minutes.

A dose-response relationship was demonstrated for the effect of EPA I on renin release at concentrations of 0.7 to 2.8 × 10⁻⁶ M with a maximum increase of about 60%. This is shown in Figure 5 (upper panel). At higher concentrations of EPA I there was a gradual decrease in response so that the rate approached control values at 2.24 × 10⁻⁵ M. After pretreatment with indomethacin the response of renin release to EPA I decreased. At a concentration of EPA I of 2.8 × 10⁻⁶ M, renin release increased by 62% and this was reduced to 12% following pretreatment with indomethacin (Fig. 5, upper panel). EPA II increased renin release in a dose-dependent manner between 1.4 × 10⁻⁵ M and 14.0 × 10⁻⁶ M. The maximum release of renin (65%) was seen at the highest concentration (Fig. 5, lower panel).

**EFFECTS OF PGE₁ AND PGF₂α**

PGE₁ in concentrations from 10⁻¹² to 10⁻⁶ M had no effect on renin release. PGF₂α in concentrations from 10⁻¹² to 10⁻⁶ M inhibited renin release in a dose-dependent manner with a maximal effect at 10⁻⁶ M (inhibition by 38%) as shown in Figure 6.

**COMPARISON OF EFFECTS ON ABSOLUTE AMOUNTS OF RENIN RELEASED**

To compare the activities of the different compounds renin release was calculated for the maximally effective concentrations of each. The results are shown in Figure 7. It appears that roughly the same degree of stimulation was obtained with PGG₂, EPA I, and EPA II (around 60%). Considering the concentrations used, EPA I was 5 times more potent than EPA II. PGG₂ probably was rapidly degraded under our experimental conditions and the stimulation achieved by addition of this compound therefore was very short-lasting. C20:4 was the most potent of the compounds studied; it stimulated renin release up to a maximum of 350% as compared to control. In all experiments in which there was pretreatment with indomethacin...
or ETA, basal as well as C20:4-stimulated or EPA I-stimulated renin release was reduced as compared to control. PGE₂ had no effect on renin release, whereas PGF₁₀ (10⁻⁶ M) inhibited renin release by about 40%.

**Discussion**

Under a variety of experimental conditions, such as a decrease in renal perfusion pressure, total renal ischemia, intravenous injection of furosemide (our unpublished results), and infusion of arachidonic acid into the renal artery, release of both prostaglandins and inhibitors. Therefore, under these conditions, in which increased renin secretion is for the most part associated with decreased renal vascular resistance, glycercol-induced acute renal failure can be reduced by PG synthesis inhibitors. However, under these conditions, in which increased renin secretion is for the most part associated with decreased renal vascular resistance, the increase in renin release may depend on the activity of the PG system in the kidney. On the other hand, the hypothesis of renin that is associated with elevated renal vascular resistance may be due to an impaired PG biosynthesis in the kidney. The present studies in vitro were performed to evaluate the possibility of a direct influence of the renal cortical PG system on renin release.

The precursor of the renal prostaglandins, arachidonic acid, elicited a dose-dependent increase in renin release from renal cortical slices up to a concentration of 4.5 × 10⁻⁶ M. The release of renin increased linearly with time for 60 minutes. Biosynthesis of prostaglandins in the renal cortex recently has been demonstrated. Therefore, a direct action of locally produced prostaglandins or PG endoperoxides on renin release seems possible. There was a curvilinear relationship between the release of renin and the concentrations of C20:4 (Fig. 3). After increasing responses at concentrations of C20:4 of 0.6 × 10⁻⁴ to 4.5 × 10⁻⁶ M, the release of renin declined at higher concentrations of C20:4. One reason for the decline in the dose-response curve could be that the C20:4 is converted to different biologically active metabolites at low and high concentrations. In the sheep vesicular gland, C20:4 is converted preferentially to PGE₂ at low concentrations and to PGF₁₀ at high concentrations. In the present experiments PGF₁₀ had an inhibitory effect on renin release (see below). Considered together, these findings could serve as one possible explanation for the curvilinear dose-response curve to C20:4. Another possibility could be the nonenzymatic formation of biologically active oxidation products at the higher concentrations of C20:4. That the effect of arachidonic acid on renin release was due to formation of prostaglandins or PG endoperoxides was supported by the finding that indomethacin and ETA inhibited basal renin release as well as the renin release stimulated by C20:4. However, indomethacin and ETA inhibited the C20:4-induced renin release only at the lower but not at the higher C20:4 concentrations. The failure of indomethacin and ETA to inhibit renin release at the higher concentrations of C20:4 could be explained by the abovementioned possibilities for relatively higher formation rates of PGF₁₀ or other oxidation products at the higher concentrations of C20:4.

To study which of the components in the PG system increased renin release we tested the effects of the natural endoperoxides PGG₂, of two stable endoperoxide analogues (EPA I and EPA II), and of PGE₂ and PGF₁₀. PGG₂ and the endoperoxide analogues increased renin release almost to the same degree. Fairly high concentrations of PGE₂ had to be used because the endoperoxide is rapidly cleaved in aqueous solutions to PGE₂, PGD₂, or PGF₁₀.

The question arises whether C20:4 elicits its effects by means of the formation of endoperoxides or by thromboxanes. In blood platelets EPA I has been shown to mimic primarily the effects of endoperoxides, whereas EPA II has been found to mimic primarily those of thromboxanes. Therefore, we used these two stable analogues as a tool to differentiate between endoperoxide- and thromboxane-induced effects of C20:4 on renin release. EPA I and EPA II had to be added to the slices in concentrations above 10⁻⁴ M to elicit a release of renin. For EPA I the effect was partially antagonized by pretreatment with inhib
domethacin. EPA I therefore might owe part of its effect to stimulation of endogenous PG biosynthesis. A similar partial antagonism of the effects of EPA I by indomethacin also was found in the platelet system. In agreement with this hypothesis is the finding that the dose-response curve of EPA I was similar to that of C20:4. Because of lack of material, the effect of EPA II was studied only at three concentrations. It is difficult therefore to make comparisons between EPA I and II. However, it seems that EPA I might be about 5 times more active than EPA II. Therefore, with regard to these compounds, our experimental system is different from the platelet system, in which EPA II is the more active analogue. Furthermore, in the platelets the main final products of the PG synthetase system are thromboxanes, whereas in the rabbit kidney the formation of PGE and PGB exceeds by far that of thromboxane B2 (unpublished data). It seems, therefore, that the increase in renin release induced by C20:4 is caused mainly by formation of the endoperoxides and not by thromboxanes.

PGE concentrations of 10^-15 to 10^-6 M had no effect on renin release in the present experiments. These concentrations were chosen because lower, rather than higher, concentrations of PGE were reported to stimulate renin release from cells of rabbit renal cortex. It might be noted that Corsini et al., in agreement with our results, found no effect of PGE on renin release from rat kidney slices. The reasons for these differences between the cell and the slice system are not clear at present. The possibility exists, however, that the activity of PGE-metabolizing enzymes is higher in the slice system than in the cell system. This, in turn, might have prevented an effect of PGE on renin release. PGE inhibited renin release in a dose-dependent manner. This finding supports a preliminary report of Dew and Michelakis, who also found a dose-related, inhibitory effect of PGE on renin release from cells of rabbit renal cortex.

Our results raise the possibility that renin release is related to the activity of the PG synthetase in the kidney cortex and more specifically to the formation of PG endoperoxides. Whether or not this occurs at the site of the juxtaglomerular apparatus will have to be explored in future experiments.

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References

Investigation of the Theory and Mechanism of the Origin of the Second Heart Sound

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SUMMARY To investigate further the origin of the second heart sound we studied human subjects, dogs, and a model in vitro of the cardiovascular system. Intra-arterial sound, pressure, and, where possible, flow and high speed cine (2,000 frames/sec) were utilized. The closure sound of the semilunar valves was of higher amplitude in the ventricles than in their respective arterial cavities. The direction of inscription of the main components of intra-arterial sound were opposite in direction to the components of intraventricular sound. Notches, representative of pressure increments, were noted on the ventricular pressure tracings and were coincident with the components of sound. The amplitude of the closure sound varied with diastolic pressure, but remained unchanged with augmentation of forward and retrograde aortic flow. Cines showed second sound to begin after complete valvular closure, and average leaflet closure rate was constant regardless of pressure. Hence, the semilunar valves, when closed, act as an elastic membrane and, when set into motion, generate compression and expansion of the blood, producing transient pressure changes indicative of sound. The magnitude of the initial stretch is related to the differential pressure between the arterial and ventricular chambers. Sound transients which follow the major components of the second sound appear to be caused by the continuing stretch and recoil of the leaflets. Clinically unexplained findings such as the reduced or absent second sound in calcific aortic stenosis and its paradoxical presence in congenital aortic stenosis may be explained by those observations.

ALTHOUGH it is generally agreed that the second heart sound is related to closure of the semilunar valves, controversy remains as to the exact mechanism. In 1915 Wiggers suggested that there is silent approximation of the semilunar valves, and that aftervibrations of the closed valves and the column of blood produce the second sound. Studies utilizing an electrical conduction device showed that aortic coaptation occurs at least 5–13 msec before the sharp apex of the aortic incisura, and studies utilizing echocardiography showed that the aortic component of the second sound occurs at or slightly after coaptation. These observations support the work of Wiggers. Others have suggested that the main vibrations of the second sound are related to the magnitude of deceleration of blood during the latter part of ventricular systole. Some attribute the second sound to vibration of the leaflets caused by the snapping together of the corpora arantii. Rushmer related the origin of the second sound to vibrations of the valvular cusps initiated by abrupt deceleration of backward-moving blood following ejection. In view of these conflicting theories, our study was undertaken to further explore the mechanism of the production of the second heart sound. The scope of this investigation is related entirely to the origin of the second sound, and does not relate to the method of sound radiation in the cardiovascular system.

Methods

Simultaneous recordings of pressure and intra-arterial sound were obtained in 10 human subjects with no evidence of valvular disease, and in one with a Bjork-Shiley tilting disk aortic valvular prosthesis. The study was approved by the appropriate human experimentation committees, and informed consent was obtained from each subject studied. Valvular disease in these 10 subjects was excluded on the basis of absence of murmurs at the chest wall and absence of a pressure gradient across the aortic valve. Pressure and sound-pressure fluctuations (which we refer to as intravascular sound) were measured with a single catheter-tip transducer in conjunction with a TCB-100 control unit which permits the recording of sound and pressure from the same transducer (Millar Instruments). The frequency response of the pressure sensor was essentially flat from 0 Hz to 10 kHz in the pressure mode. The audio mode low frequency cutoff, when used with 10-MΩ loading, was 6 dB at 40 Hz, 3 dB at 50 Hz, and flat above 90 Hz. The phase lag of the single-gauge transducer was 90° at 35 kHz, which is equivalent to a time delay of approximately 7 μsec. Tests showed no artifacts or apparent sound production by snapping or tapping the body of the catheter or motion of the tip of the transducer. Tests...
Stimulation of renin release from rabbit renal cortex by arachidonic acid and prostaglandin endoperoxides.

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