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STIMULATION OF RENIN RELEASE BY 6-OXO-PROSTAGLANDIN E_1 AND PROSTACYCLIN

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1 Renin release induced by 6-oxo-prostaglandin E_1 (6-oxo-PGE_1) was compared to release in response to prostacyclin (PGI_2) and 6-oxo-$PGF_{1\alpha}$ in slices of rabbit renal cortex.

2 Krebs-Ringer medium bathing slices of renal cortex was collected for renin assay after four successive 20 min intervals (periods I–IV). Renin release did not increase during periods I to IV in untreated slices. Agonists were added, only once, at the beginning of period III. Between periods III and IV, the incubation solution was aspirated and replaced with fresh medium.

3 PGI_2 increased renin release during period III while 6-oxo-PGE_1 stimulated release during periods III and IV. 6-oxo-PGE_1 stimulated renin release (24%–74%) in concentrations ranging from 1–33 μM while PGI_2 stimulated release at 10 μM (60%) but not at 5 μM. 6-oxo-$PGF_{1\alpha}$, 10 μM, did not release renin during period III (period III, 9%), but caused a small rise in period IV (29%).

4 6-oxo-PGE_1, unlike PGI_2, was stable under the incubation conditions (pH 7.4, 37°C) as indicated by recovery of undiminished platelet anti-aggregatory material after 20 min.

5 In the rabbit kidney, activity of 9-hydroxyprostaglandin dehydrogenase was greatest in the cortex and negligible in the papilla, corresponding to the zonal distribution of renin.

6 The prominent and sustained *in vitro* renin releasing effect of 6-oxo-PGE_1, as well as the cortical localization of enzyme activity capable of generating this stable prostacyclin metabolite, suggest that formation of 6-oxo-PGE_1 may contribute to PGI_2-induced renin release and may explain the delayed stimulation caused by 6-oxo-$PGF_{1\alpha}$.

Introduction

The regulation of renin release involves a prostaglandin mechanism. Arachidonic acid, the precursor of bisenoic prostaglandins, stimulates renin secretion from the kidney, an effect inhibited by indomethacin (Larsson, Weber, & Änggard, 1974). Of the known arachidonic acid metabolites, prostacyclin (PGI_2) is considered to be the most likely mediator of the prostaglandin-dependent renin releasing mechanism (Oates, Whorton, Gerkens, Branch, Hollifield, & Frölich, 1979). However, it has been suggested that metabolism of PGI_2 and, perhaps, 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$), in the liver and possibly other tissues may result in the formation of 6-oxo-PGE_1 via the 9-hydroxyprostaglandin dehydrogenase (9-OH PGDH) pathway (Wong, Malik, Desiderio, McGiff, & Sun, 1980). The latter enzyme, which can convert F-type prostaglandins to the E-series equivalent, has also been identified in human platelets (Wong, Lee, Chao, Reiss, & McGiff, 1980a) and in rat (Pace-Asciak, 1975) and rabbit kidneys (Moore & Hoult, 1978). Like PGI_2, 6-oxo-PGE_1 can inhibit the aggregation of platelets (Wong, McGiff, Sun, & Lee, 1979) and reduce blood pressure and renal (Quilley, Wong & McGiff, 1979), mesenteric (Feigen, Chapnick, Hyman, King, Marascalco & Kadowitz, 1980) and pulmonary vascular resistances (Hyman & Kadowitz, 1980). While the present study was in progress, Jackson and co-workers (Jackson, Herzer, Zimmerman, Branch, Oates & Gerkens, 1981) showed that 6-oxo-PGE_1 stimulated renin secretion from the dog kidney *in situ* at rates of infusion into the renal artery less than those required to demonstrate a prostacyclin effect. In the present study, we examined the possibility that 6-oxo-PGE_1 causes renin release from renal slices, a preparation isolated from haemodynamic, neural and blood-borne factors. The effects of 6-oxo-PGE_1 were compared to those of isoprenaline, PGI_2 and 6-oxo-$PGF_{1\alpha}$. The decay of human platelet anti-aggregatory activity was used to assess the stability of PGI_2 and 6-oxo-PGE_1. In addition, we determined the distribution of 9-OH PGDH activity in cortical, medullary and papillary zones of rabbit kidney.

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Methods

Renal cortical slices

New Zealand white male rabbits (2.6–3.5 kg) were anaesthetized with sodium pentobarbitone (25 mg/kg, i.v.). After excising and decapsulating the kidney, two slices (approximately 0.5 mm thick) were obtained from the superficial cortex using a Stadie-Riggs microtome. Each slice was subdivided and the sections added to flasks (1–2 tissues per flask, 10–30 mg dry wt.) containing 5 ml Krebs-Ringer solution (mM): NaCl 118, KCl 4.75, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.2, $NaHCO_3$ 24.3, with 1 g/l dextrose and 100 μg/ml bovine serum albumin (BSA) (KR-BSA). All flasks were placed in a shaking water bath at 37°C and the tissues gassed with 95% O_2/5% CO_2 saturated with water vapour. After 20 min of preincubation, the medium was aspirated and the tissues rinsed with 5.0 ml fresh KR-BSA solution. The rinse solution was discarded and the slices incubated with 5.0 ml fresh KR-BSA solution for four successive 20 min periods (I, II, III and IV). The first two periods were employed to indicate basal release, and test agents and vehicle solutions were added in a maximum volume of 10 μl at the start of period III (the experimental period). At the conclusion of period III, all tissues were rinsed quickly with an additional 5 ml KR-BSA solution before starting IV, which served as a 'recovery' period. In those experiments using isoprenaline as the test agent, ascorbic acid (6.0 mM) was included in the medium, as an anti-oxidant, during all periods. Ascorbic acid, alone, did not influence renin release. Responses to each concentration were tested using tissues from at least two rabbits. Aliquots of slice incubation medium withdrawn after each period were centrifuged at 4°C and used, without freezing, to generate angiotensin I from homologous substrate. The method of sampling the slice medium has been used to elucidate the effect of catecholamines on renin release (Aoi, Wade, Rosner & Weinberger, 1974).

Renin assay

Samples (100 μl) of the medium were incubated in a shaking water bath at 37°C for 1 h with 500 μl plasma from nephrectomized rabbits (pooled from 4 rabbits bilaterally nephrectomized 24 h before bleeding) in the presence of disodium edetate (EDTA) (3.5 mM), PMSF (3.0 mM), Tris-maleate pH 7.4 buffer (200 mM) and BSA (3 mg/ml). Angiotensin I was generated at pH 7.4 in order to reduce the contribution of renin-like enzymes with more acidic pH optima. Reaction mixture volume was adjusted to 1.0 ml with 100 mM NaH_2PO_4–Na_2HPO_4, pH 7.4, buffer. The reaction was stopped by freezing (− 20°C), and samples were stored at − 20°C until assayed (less than 2 weeks).

Linearity of angiotensin I yield versus enzyme concentrations, under these conditions, was established using both a purified commerical preparation of lyophilized hog renin (Schwartz-Mann) and the KR-BSA medium from actual rabbit renal slice experiments. For the former, 5 mg of lyophilized powder of hog renal renin (mol. wt. 42,000–49,000), was applied to a 22 × 2.3 cm Sephadex G75 column which had been equilibrated against 100 mM NaH_2PO_4–Na_2HPO_4, pH 6.0, buffer. Elution was performed with the same buffer and 10 ml fractions were collected. The protein was eluted in one peak, 85% of the total being recovered within the first fraction after the void volume (approximately 32 ml). Different volumes of the fraction showing peak activity were incubated for 1 h with nephrectomized rabbit plasma under the reaction conditions described above. Generation of angiotensin I was linear throughout the range tested (i.e., up to 500 ng/h); angiotensin I was not detected in the substrate blank (no eluate).

Similarly, linear generation of angiotensin I was evident when different volumes of fresh slice medium, expected to have high renin activity (slices exposed to isoprenaline) were incubated under the same conditions with nephrectomized rabbit plasma. Samples of slice medium incubated in the absence of nephrectomized rabbit plasma (enzyme blank) do not contain measurable amounts of angiotensin I (or material cross-reactive with angiotensin I).

After collecting each pool of nephrectomized rabbit plasma, renin substrate concentration was estimated by kinetic assay in the presence of a high concentration of partially purified hog renin; i.e. 95 μl of the Sephadex G-75 eluate (Figure 1). In each assay a plateau level of 2200–2640 ng angiotensin I equivalents/ml was reached within 1 to 2 h incubation and maintained for at least 7 h, indicating inhibition of the activity of angiotensinases. Further, greater than 92% recovery was obtained when 2 ng of angiotensin I was added to the reaction mixture as an internal standard. The estimated substrate concentration compares favourably with the value of 2900 ng/ml found by Weber, Held, Uhlich & Eigler (1975) who used 24 h post-nephrectomy rabbits and partially purified rabbit renin.

Angiotensin I generation was determined with the Squibb angiotensin I Immutope Kit. The interassay coefficient of variation for 8 consecutive radioimmunoassays was 2.6% and displacement of radioactivity by a 10 pg angiotensin I standard, tested in 6, ranged from 4–7% of maximum binding. Values termed 'renin release' were corrected for dilution and

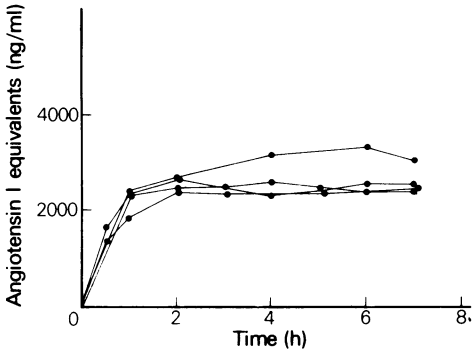

Figure 1 Time course of angiotensin I generated from nephrectomized rabbit plasma and partially purified hog renin. Renin substrate concentration, expressed as ng angiotensin I equivalents/ml plasma, is plotted on the ordinate for separate kinetic assays ($n = 4$ assays). Each assay was performed using plasma pooled from 4 animals bilaterally nephrectomized 24 h before bleeding. Details of reaction conditions are given under Methods. Angiotensin I was determined by radioimmunoassay.

expressed as ng angiotensin I generated ml^{-1} slice medium h^{-1} mg^{-1} dry wt. The statistical significance of changes in renin release was evaluated using Student's paired t test, comparing the experimental period (III) or the recovery period (IV) to the control period (II). Results are presented as mean ± s.e. and $P < 0.05$ was considered to be statistically significant.

Platelet aggregation experiments

We assessed the stability of PGI_2 and 6-oxo-PGE_1 in the incubation medium used for our slice experiments (in the absence of tissue) by following inhibition of platelet aggregation after different time intervals. Aggregation tests were performed in triplicate for each prostaglandin at a final concentration in KR-BSA solution of 10 μM. A single test was also carried out for 100 μM 6-oxo-PGE_1. After the final sampling in each series, the pH was verified to be 7.35–7.40. As in the renin studies, 10 μl of vehicle containing either 6-oxo-PGE_1 or PGI_2 was added to 5 ml KR-BSA solution which had been incubated and gassed for 20 min to fix the temperature and pH.

Samples (20 μl) were withdrawn after 15 s, 10 min, and 20 min, and immediately added to ice-cold 50 mM Tris buffer (pH 9.3 at 5°C, total volume 1 ml). These dilutions, as well as blanks, were then tested for inhibition of aggregation of human platelets by adding small aliquots containing 0.5–6.8 ng, calculated for zero time, to 0.5 ml platelet-rich plasma 1 min before addition of adenosine diphosphate (ADP, 5 μM). The procedures used for the platelet aggregation experiments have been described in detail elsewhere (Wong *et al.*, 1979).

Zonal distribution of 9-hydroxyprostaglandin dehydrogenase activity

Rabbit kidneys were bisected along the longitudinal axis. The renal papilla was severed at the junction with the inner zone of the medulla. The cortex was trimmed from the outer medulla avoiding overlapping regions; the junctional zone was then removed from the remaining block of medullary tissue and discarded. After homogenization in Tris 50 mM buffer (pH 8.4), samples were centrifuged for 30 min at 7,000 *g* to remove cell debris. The supernate was centrifuged at 100,000 *g* for 60 min in a Beckman model L-75 ultracentrifuge using a type W-28 rotor. 9-Hydroxyprostaglandin dehydrogenase activity was assayed according to the method of Tai & Yuan (1977). Briefly, the assay mix contained nicotinamide adenine dinucleotide (NAD^+, 4 mM) either [9-^{3}H]-$PGF_{2\alpha}$ or 6-oxo-$PGF_{1\alpha}$ (10,000 d/min), lactic dehydrogenase (50 μg), sodium pyruvate (5 mM), EDTA (1 mM), Tris buffer (50 mM, pH 8.4) and an aliquot of high speed supernate (final volume = 1 ml). The reaction was started by addition of substrate and ended by addition of dextran-coated charcoal suspension. After standing for 5 min at room temperature, the reaction mixture was centrifuged at 1,000 *g* for 5 min. An aliquot of supernate was transferred to 10 ml Liquiscint (National Diagnostics) and counted in a Beckman LS7500 liquid scintillation system. Results were expressed as pmol of prostaglandin oxidized mg^{-1} protein h^{-1}. Protein concentration was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

Drugs

(−)-Isoprenaline (mol. wt. 211.2, Sigma), purified hog renin (Schwartz-Mann) and all prostaglandins (Upjohn) were stored dessicated as the solid form at −20°C. Solutions were freshly prepared on the day of the experiment. Prostacyclin sodium salt (mol. wt. 374.45) was diluted to 1.76 mg/ml, expressed as the salt, with ice-cold Tris 50 mM, pH 9.3, buffer. 6-oxo-PGE_1 (mol. wt. 352) was dissolved in dry acetone (17.0 or 56.1 mg/ml) and diluted ten or one-hundred fold with Krebs-Ringer solution. 6-oxo-$PGF_{1\alpha}$ (mol. wt. 370.47) was prepared in Krebs-Ringer solution (final concentration = 1.84 mg/ml). A 10.1 μg/ml solution of (−)-isoprenaline was prepared in KR-BSA ascorbic acid (6 mM). Bovine albumin, fraction V (reagent grade) was obtained from Miles Laboratories. Test agents were stored briefly on ice before addition to the incubation flasks.

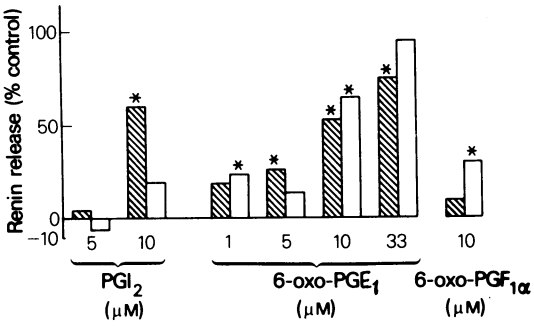

Figure 2 Effects of prostacyclin (PGI_2), 6-oxo-prostaglandin E_1 (6-oxo-PGE_1) and 6-oxo-$PGF_{1\alpha}$ on renin release from rabbit renal cortical slices. Renal slices were incubated in Krebs-Ringer solution for 4 successive 20 min periods (I–IV) and medium was collected for renin assay after each period. Renin release did not increase during periods I–IV in untreated slices. Prostaglandins were added, only once, at the beginning of period III; the final concentrations achieved are indicated. Between periods III and IV, the incubate was aspirated and replaced with fresh medium after rinsing. The same data as are given in Table 1 are depicted here but renin release during periods III (diagonally-hatched columns) and IV (open columns) is expressed as a percentage of the control period II value. Statistical significance was determined by Student's paired *t* test; $*P<0.05$ compared to renin release during the control period.

Results

Mean rates of renin release from cortical slices during control and experimental periods are given in Table 1 and Figure 2. Linear release of renin is implicit in this experimental design as indicated by the stability of basal renin release during the first two periods before test agents were added (Table 1). The addition of 10 μl of Tris 50 mM (pH 8.8 at 37°C) vehicle ($n=2$) for PGI_2 or KR-acetone (9 : 1 v/v) vehicle ($n=2$) for 6-oxo-PGE_1 did not affect renin release. These results have been pooled and designated the control group (Table 1). Mean renin release for this group was 1.26 ± 0.32 and 1.17 ± 0.28 ng ml^{-1} h^{-1} mg^{-1} at the end of periods II and III, respectively. Agonists were always added, only once, at the beginning of period III. Between periods III and IV, the incubation solution was aspirated and replaced with new medium after rinsing. All of the agonists tested, with the exception of 6-oxo-$PGF_{1\alpha}$, were found to stimulate the release of renin during period III (Table 1). However, it should be noted that rinsing, after completion of period III, resulted in variable changes during period IV depending on the agonist used. Isoprenaline, added after completion of period II, resulted in an increase in renin released during period III ($P<0.02$) (Table 1), an effect which sometimes persisted throughout period IV despite rinsing between periods III and IV as indicated. For isoprenaline, renin release during period IV increased

Table 1 Mean rates of renin release from rabbit renal cortical slices as affected by isoprenaline, prostacyclin (PGI_2), 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) and 6-oxo-PGE_1

						Experimental	
	Concentration		*Basal*		*Agonist*	*III*	*IV*
Agonists	(μM)	n	*I*	*II*	*added*	*Rinse*	
Control		5	1.33	1.26		1.17	1.17
			(.34)	(.32)		(.28)	(.29)
Isoprenaline	0.1	5	1.19	1.25		2.12†	2.34
			(.09)	(.22)		(.41)	(.61)
PGI_2	5	4	1.40	1.58		1.65	1.47
			(.44)	(.22)		(.24)	(.38)
	10	6	1.33	1.41		2.26†	1.68
			(.27)	(.25)		(.48)	(.37)
6-oxo-$PGF_{1\alpha}$	10	6	1.54	1.56		1.70	2.01†
			(.22)	(.14)		(.12)	(.13)
6-oxo-PGE_1	1	6	1.36	1.44		1.71	1.78†
			(.27)	(.21)		(.28)	(.28)
	5	5	1.23	1.10		1.39*	1.24
			(.14)	(.15)		(.15)	(.17)
	10	5	1.29	1.77		2.70*	2.91*
			(.35)	(.66)		(.87)	(1.03)
	33	4	1.30	1.29		2.25*	2.52
			(.34)	(.34)		(.59)	(.81)

n denotes number of slice incubations. Renin release in ng $ml^{-1}h^{-1}mg^{-1}$ dry wt. is expressed as mean (s.e.mean).
* † denotes statistically significant changes when compared with period II values; $*P<0.05$; $†P<0.02$.

further in 3 experiments, but fell to control levels in the other 2. When propranolol, 200 μM, was added to the flask ($n=3$) isoprenaline did not increase renin release (not shown).

Prostacyclin, 10 μM, stimulated renin release by 60% ($P<0.02$) but did not affect renin release at 5 μM (Figure 2). Further, unlike 6-oxo-PGE$_1$, the effects of PGI$_2$, on renin release did not persist after the rinse between periods III and IV. Two important effects of 6-oxo-PGE$_1$ were apparent; the threshold concentration which stimulated renin release was lower than that of PGI$_2$ and the effects usually persisted through period IV (Figure 2) in a manner which seemed to be dose-related. The renin releasing threshold concentration of 6-oxo-PGE$_1$ was between 1 and 5 μM as the former concentration of 6-oxo-PGE$_1$ effected a 24% increase above control in period IV, and the latter a 26% increase in period III. In response to 10 μM of 6-oxo-PGE$_1$ renin release increased above control values by 52% and 64% during periods III and IV, respectively (Figure 1). The highest concentration of 6-oxo-PGE$_1$ tested, 33 μM, increased renin release by 74% during period III ($P<0.05$); during period IV renin release increased further in three of four experiments.

In contrast to PGI$_2$ or 6-oxo-PGE$_1$, 6-oxo-PGF$_{1\alpha}$ (Figure 2) did not affect renin release during period III; however, renin release was increased during period IV ($P<0.02$) when compared to the basal period II value or to period IV of control incubations (Table 1, Figure 2).

For the platelet aggregation tests, aliquots (7–29 μl) of Tris buffer dilutions (containing 0.5–6.8 ng of either PGI$_2$ or 6-oxo-PGE$_1$, amount estimated at zero time) were added to aggregometer cuvettes 1 min before induction of platelet aggregation by ADP (5 μM). Most of the platelet anti-aggregatory activity of prostacyclin was lost within 10 min of incubation (Figure 3a). Percentage inhibition of platelet aggregation by samples from incubates containing PGI$_2$ (0.5–0.7 ng), performed in triplicate, was 91±2%, 29±6% and 4±7% after 15 s, 10 min and 20 min, respectively. In contrast, 6-oxo-PGE$_1$ retained its platelet anti-aggregatory activity after 20 min of incubation. The percentage inhibition of platelet aggregation caused by addition of samples from incubates containing 6-oxo-PGE$_1$ (1.4–2.0 ng), performed in triplicate, was 53±8%, 59±7%, and 51±11% after 15 s, 10 min, and 20 min, respectively. Similarly, 6-oxo-PGE$_1$ (6.8 ng) caused inhibition of ADP-induced platelet aggregation without loss of activity when tested at 15 s, 10 min, and 20 min (Figure 3b). Thus, these platelet aggregation experiments indicate that 6-oxo-PGE$_1$ is considerably more stable than prostacyclin under the incubation conditions used for studying renin release from cortical slices.

In the rabbit kidney, 9-OH PGDH activity was found to be greatest in the high speed supernate obtained from the cortex, the zone of highest renin activity (Bing, Eskildsen, Faarup, & Frederiksen, 1967). The reaction was time and protein

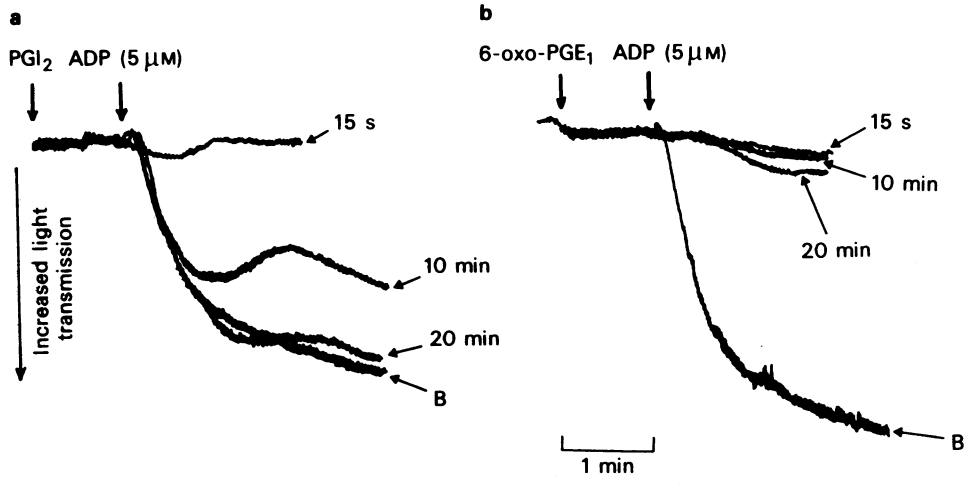

Figure 3 Platelet anti-aggregatory effects of samples obtained at various time intervals after addition of either prostacyclin (PGI$_2$) (a) or 6-oxo-prostaglandin E$_1$ (6-oxo-PGE$_1$) (b) to Krebs-Ringer medium (0.01% BSA). Prostaglandins were added after the medium had been incubated and gassed to fix the pH and temperature (pH 7.35–7.40, 37°C). Small aliquots were removed after the indicated time periods and added to 50 mM Tris buffer (pH 9.3 at 5°C). The Tris dilutions were then tested for inhibition of aggregation of human platelets by adding to 0.5 ml platelet-rich plasma 1 min before addition of ADP (5 μM) as indicated. Aggregation recordings demonstrate inhibitory effects of PGI$_2$ (0.7 ng, calculated for zero time, a) and 6-oxo-PGE$_1$ (6.8 ng, calculated for zero time, b). B denotes tracings obtained with vehicle blanks.

concentration-dependent with a pH optimum of 8.5 to 9.0 and a requirement for NAD^+ as reported previously for 9-OH PGDH purified from rat kidney (Yuan, Tai & Tai, 1980). Activity in the medulla was approximately one-fifth that in cortex, while negligible oxidation was found in the cytosol of the papilla: using [9-^{3}H]-$PGF_{2\alpha}$ as substrate, cortex 3.2, medulla 0.69, and papilla < 0.10 pmol mg^{-1} protein h^{-1}. The high speed supernate of the cortex also oxidized 6-oxo-$PGF_{1\alpha}$ to 6-oxo-PGE_1; specific activity was 4.4 pmol mg^{-1} protein h^{-1}.

Discussion

PGI_2 has been considered to be the most likely metabolite of arachidonic acid to mediate the release of renin (Oates *et al.*, 1979). However, 6-oxo-PGE_1 can produce an equal or greater response in the same concentration range. PGI_2 reportedly stimulated the release of renin from rabbit cortical slices in concentrations ranging from 0.1 to 10 μM (Whorton, Misono, Hollifield, Frolich, Inagami & Oates, 1977). Because of the different reaction conditions used by these investigators to generate angiotensin I, it is difficult to attribute their finding of a lower threshold for PGI_2 to any specific difference in experimental design. For example, the lower temperature (25°C) at which the slices were incubated may have contributed to the increased ability of PGI_2 to release renin when compared to our study which was conducted at 37°C. The half-life of prostacyclin in aqueous solution may increase 3 to 4 fold when the temperature is lowered from 37.5 to 25°C (Johnson, Morton, Kinner, Gorman, McGuire, Sun, Whittaker, Bunting, Salmon, Moncada & Vane, 1976; Cho & Allen, 1978). Under our experimental conditions, PGI_2 did not increase renin release at a concentration of 5 μM whereas 6-oxo-PGE_1 did. While the concentrations of 6-oxo-PGE_1 and PGI_2 required to release renin are relatively high when compared to isoprenaline, this consideration must be related to the level of activity of metabolic enzymes and transport mechanisms controlling intracortical distribution, as well as to the background levels of endogenous prostaglandins. The latter could have affected the sensitivity of our preparation to exogenous prostaglandins. Nonetheless, we elected not to carry out this study with indomethacin treatment because of the known effects of cyclo-oxygenase inhibitors on the enzyme, 9-OH PGDH (Yuan *et al.*, 1980).

Changes in the intracellular levels of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) can affect renin release by the kidney and may represent a final common pathway for the expression of those mechanisms affecting renin secretion. Both PGI_2 and 6-oxo-PGE_1 have been shown to stimulate adenylate cyclase activity. In membrane fractions prepared from rat and rabbit kidney cortex (Rapp, Zenser & Davis, 1981), as well as in human lymphocytes and platelets (Wong *et al.*, 1979; Mastacchi, Fadda, Tomasi & Barnabei, 1980), 6-oxo-PGE_1 caused elevation of cyclic AMP levels. Thus, the stimulatory effect of 6-oxo-PGE_1 on renin release may depend on elevation of intracellular cyclic AMP levels. It is noteworthy that Rapp *et al.* (1981) found 8 μM to be the lowest concentration of 6-oxo-PGE_1 effective in stimulating adenylate cyclase activity in membrane fractions prepared from rat renal cortex, a concentration remarkably similar to the threshold concentration which we found to stimulate renin release.

Mention should be made of the variability of values for basal renin release (Table 1). Although basal renin release during periods I and II was generally stable for each individual incubation, the absolute levels of renin release exhibited considerable variation between incubations, even when corrected for slice weight. The reason(s) for apparent differences in basal renin release among the various test groups is not clear but may be related to small differences in age or to seasonal variation or to adaptation to captivity. Similar variability was evident in the *in vitro* experiments of Weinberger, Aoi & Henry (1975) which were similar in design to those reported here.

In the present investigation, continued stimulation of renin release was observed in the recovery period (IV) in response to isoprenaline and to 6-oxo-PGE_1 (Table 1). Weinberger and coworkers have also commented on the tendency for the stimulation of renin release from rat kidney slices to persist after high concentrations of adrenaline (Aoi *et al.*, 1974) despite replacement of the incubating medium. The failure to 'wash out' the 6-oxo-PGE_1 response may indicate sequestration at an intracellular site. As an alternative or additional mechanism, the incomplete recovery may reflect a prolonged action on intracellular levels of cyclic AMP.

Biological activities of 6-oxo-PGE_1 closely resembling those of PGI_2 have been described; viz, the two substances inhibited platelet aggregation (Wong *et al.*, 1979), reduced blood pressure (Quilley *et al.*, 1979), and caused vasodilatation in all regional vascular beds tested (Quilley *et al.*, 1979; Feigen *et al.*, 1980; Hyman & Kadowitz, 1980). In a recent study, Jackson *et al.* (1980) showed that 6-oxo-PGE_1 was a more potent renin secretagogue and renal vasodilator than PGI_2. The present study is consistent with a direct stimulatory effect of 6-oxo-PGE_1 on the renin secreting cells. Relative to the changes caused by PGI_2, 6-oxo-PGE_1-induced renin release occurred at lower concentrations and was more sustained.

The identity of the arachidonic acid metabolite responsible for stimulating renin secretion has not been definitely established, although it has been proposed that PGI_2 is the physiological mediator (Oates *et al.*, 1979). This proposal was based, in part,

on the results of the *in vitro* experiments of Whorton *et al.* (1977). However, the stimulation of renin released by PGI_2 in the cortical slice preparation of Whorton *et al.* (1977) was time-dependent; i.e., the response was essentially linear for at least 30 min of incubation. It is interesting to note that after addition of PGI_2 the incubating medium was not changed. The long duration of this response was unexpected as PGI_2 is rapidly hydrolyzed to 6-oxo-$PGF_{1\alpha}$ in aqueous solution at physiological pH and temperature (Johnson *et al.*, 1976). Moreover, there are reports in the literature of platelet anti-aggregatory effects of PGI_2 lasting long after its chemical half-life (Higgs, Moncada & Vane, 1977; Szczeklik, Gryglewski, Nizankowska, Nizankowski & Musial, 1978) and continued elevation of platelet cyclic AMP levels despite neutralization with anti-PGI_2 antibodies (Smith, Ogletree, Lefer & Nicolaou, 1978).

Increasing evidence suggests that in some *in vitro* systems or even *in vivo* prostacyclin may be subject to biotransformation to an active product rather than rapid non-enzymatic inactivation. A recent study by Gimeno, Sterin-Borda, Borda, Lazzari & Gimeno (1980) supports this idea and may have a direct bearing on our finding of increased renin release in period IV following exposure to 6-oxo-$PGF_{1\alpha}$. These authors exploited the differential sensitivity of the bovine coronary artery strip preparation to 6-oxo-PGE_1 (contraction) and PGI_2 (relaxation) in an investigation of the biotransformation of PGI_2 and 6-oxo-$PGF_{1\alpha}$ by human plasma. Incubation of PGI_2 for 60–150 min periods resulted in the generation of material which contracted the bovine coronary artery strip whereas, before 30 min, only material having PGI_2-like relaxant activity was recovered. However, Gimeno *et al.* (1980) ascribed the ability to transform PGI_2 to a plasma factor, as platelet-poor plasma as well as platelet-rich plasma showed this effect. In contrast, Hoult, Lofts & Moore (1981), who demonstrated prolongation of platelet anti-aggregatory activity and enhanced spasmogenic effects after incubation of prostacyclin in human plasma, related these effects to a platelet factor. It should be noted that the enzyme 9-OH PGDH has been isolated and characterized in human platelets (Wong *et al.*, 1980a). Further, Hoult *et al.* (1981) have tentatively identified the product of PGI_2 responsible for these effects in platelets as 6-oxo-PGE_1, based on chromatographic behaviour and musculotropic properties. It should be emphasized that Gimeno *et al.* (1980) found no activity when 6-oxo-$PGF_{1\alpha}$ was initially tested on the coronary arterial strips, yet tone increased following incubation of 6-oxo-$PGF_{1\alpha}$ in plasma for 120 min. We speculate that in some tissues such as platelets, liver and kidney, 6-oxo-$PGF_{1\alpha}$ may be subject to slow and limited entry into the cytosol, an obligatory step before metabolism by 9-OH PGDH. Platelet membranes have been shown to possess high affinity binding sites for PGI_2, PGE_1 and PGE_2 but not for 6-oxo-$PGF_{1\alpha}$ (Schafer, Cooper, O'Hara & Handin, 1979). Presumably the absence of receptors indicates that the platelet does not have an uptake/binding mechanism for 6-oxo-$PGF_{1\alpha}$.

Little information is available relating to the formation of 6-oxo-PGE_1 *in vivo*. Stoff, Stemerman, Steer, Salzman & Brown (1980) have described in patients with Bartter's syndrome a plasma factor which appears to be responsible for a defect in platelet aggregation associated with abnormally high cyclic AMP levels in platelet-rich plasma. They propose that this factor is a prostaglandin metabolite as the defective aggregation and elevated cyclic AMP levels are sensitive to indomethacin treatment. The authors stress that the stability of the plasma factor argues against prostacyclin. In a preliminary communication, this stable factor has been tentatively identified as 6-oxo-PGE_1 by its mobility on high pressure liquid chromatography (Clive, Leone, MacIntyre, Brown, Salzman & Stoff, 1981). Using the decay of human platelet anti-aggregatory activity as an index of stability in the buffer used for the slice experiments, we found no evidence for degradation of 6-oxo-PGE_1 after 20 min of incubation at pH 7.4, 37°C. In contrast, the anti-aggregatory effect of PGI_2 was reduced by more than one half after 10 min under these conditions.

Our finding of a gradient of 9-OH PGDH activity from cortex to papilla is an extension of previous work by Moore & Holt (1978), who described higher 9-OH PGDH activity in rabbit cortex relative to medulla using [9-^{3}H]-$PGF_{2\alpha}$ as substrate. In the present study, conversion was observed with both [9-^{3}H]-$PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$. Thus, the intrarenal distribution of enzyme activity capable of generating 6-oxo-PGE_1 corresponds to the zonal distribution of renin. Taken together, the present results suggest that stimulation of renin release previously attributed to PGI_2 may have resulted from conversion of either prostacyclin or 6-oxo-$PGF_{1\alpha}$ to 6-oxo-PGE_1.

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References

AOI, W., WADE, M.B., ROSNER, D.R. & WEINBERGER, M.H. (1974). Renin release by rat kidney slices in vitro: effects of cations and catecholamines. *Am. J. Physiol.*, **227**, 630–634.

BING, J., ESKILDSEN, P.C., FAARUP, P. & FREDERIKSEN, O. (1967). Location of renin in kidneys and extrarenal tissues. *Circulation Res.*, **XX, XXI**, 11–3 and 11–13.

CLIVE, D.M., LEONE, D., MACINTYRE, D.E., BROWN, R.S., SALZMAN, E. & STOFF, J.S. (1981). A plasma factor resembling 6-oxo-PGE_1 inhibits platelet aggregation in Bartter's syndrome and normal man. *Clin. Res.*, **29**, 332A (Abstract).

CHO, M.J. & ALLEN, M.A. (1978). Chemical stability of prostacyclin (PGI_2) in aqueous solutions. *Prostaglandins*, **15**, 943–954.

FEIGEN, L.P., CHAPNICK, B.M., HYMAN, A.L., KING, L., MARASCALCO, B. & KADOWITZ, P.J. (1980). Peripheral vasodilator effects of prostaglandins: comparison of 6-keto-prostaglandin E_1 with prostacyclin and escape from prostaglandin E_2 in the mesenteric vascular bed. *J. Pharmac. exp. Ther.*, **214**, 528–534.

GIMENO, M.F., STERIN-BORDA, L., BORDA, E.S., LAZZARI, M.A. & GIMENO, A.L. (1980). Human plasma transforms prostacyclin (PGI_2) into a platelet antiaggregatory substance which contracts isolated bovine coronary arteries. *Prostaglandins*, **19**, 907–916.

HIGGS, G.A., MONCADA, S. & VANE, J.R. (1977). Prostacyclin (PGI_2) inhibits the formation of platelet thrombi induced by adenosine diphosphate (ADP) *in vivo*. *Br. J. Pharmac.*, **61**, 137P.

HOULT, J.R.S., LOFTS, F.J. & MOORE, P.K. (1981). Stability of prostacyclin in plasma and its transformation by platelets to a stable spasmogenic product. *Br. J. Pharmac.*, **73**, 218P.

HYMAN, A.L. & KADOWITZ, P.J. (1980). Vasodilator actions of prostaglandin 6-keto-E_1 in the pulmonary vascular bed. *J. Pharmac. exp. Ther.*, **213**, 468–472.

JACKSON, E.K., HERZER, W.A., ZIMMERMAN, J.B., BRANCH, R.A., OATES, J.A. & GERKENS, J.F. (1981). 6-Keto-prostaglandin E_1 is more potent than prostaglandin I_2 as a renal vasodilator and renin secretagogue. *J. Pharmac. exp. Ther.*, **216**, 24–27.

JOHNSON, R.A., MORTON, D.R., KINNER, J.H., GORMAN, R.R., MCGUIRE, J.C., SUN, F.F., WHITTAKER, N., BUNTING, S., SALMON, J., MONCADA, S. & VANE, J.R. (1976). The chemical structure of prostaglandin X (prostacyclin). *Prostaglandins*, **12**, 915–928.

LARSSON, C., WEBER, P. & ÄNGGÅRD, E. (1974). Arachidonic acid increases and indomethacin decreases plasma renin activity in the rabbit. *Eur. J. Pharmac.*, **28**, 391–394.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

MASTACCHI, R., FADDA, S., TOMASI, V. & BARNABEI, O. (1980). The effect of 6-keto-prostaglandin E_1 on human lymphocyte cAMP levels. *Prostaglandins and Medicine*, **5**, 487–494.

MOORE, P.K. & HOULT, J.R.S. (1978). Prostaglandin metabolism in rabbit kidney: Identification and properties of a novel prostaglandin 9-hydroxydehydrogenase. *Biochim. biophys. Acta*, **528**, 276–287.

OATES, J.A., WHORTON, R., GERKENS, J.F., BRANCH, R.A., HOLLIFIELD, J.W. & FRÖLICH, J.C. (1979). The participation of prostaglandins in the control of renin release. *Fedn Proc.*, **38**, 72–74.

PACE-ASCIAK, C. (1975). Prostaglandin 9-hydroxydehydrogenase activity in the adult rat kidney. *J. biol. Chem.*, **250**, 2789–2794.

QUILLEY, C.P., WONG, P. Y-K & MCGIFF, J.C. (1979). Hypotensive and renovascular actions of 6-keto-prostaglandin E_1, a metabolite of prostacyclin. *Eur. J. Pharmac.*, **57**, 273–276.

RAPP, N.S., ZENSER, T.V. & DAVIS, B.B. (1981). 6-Ketoprostaglandin E_1 stimulation of rat and rabbit renal adenylate cyclase-cyclic AMP systems. *Biochim. biophys. Acta*, **673**, 163–169.

SCHAFER, A.I., COPPER, B., O'HARA, D. & HANDIN, R.I. (1979). Identification of platelet receptors for prostaglandin I_2 and D_2. *J. biol. Chem.*, **254**, 2914–2917.

SMITH, J.B., OGLETREE, M.L., LEFER, A.M. & NICOLAOU, K.C. (1978). Antibodies which antagonise the effects of prostacyclin. *Nature*, **274**, 64–65.

STOFF, J.S., STEMERMAN, M., STEER, M., SALZMAN, E. & BROWN, R.S. (1980). A defect in platelet aggregation in Bartter's syndrome. *Am. J. Med.*, **68**, 171–180.

SZCZEKLIK, A., GRYGLEWSKI, R.J., NIZANKOWSKA, E., NIZANKOWSKI, R. & MUSIAL, J. (1978). Pulmonary and anti-platelet effects of intravenous and inhaled prostacyclin in man. *Prostaglandins*, **16**, 651–659.

TAI, H-H & YUAN, B. (1977). A simple and sensitive assay for 9-hydroxyprostaglandin dehydrogenase. *Anal. Biochem.*, **78**, 410–422.

WEBER, P., HELD, E., UHLICH, E. & EIGLER, J.O.C. (1975). Reaction constants of renin in juxtaglomerular apparatus and plasma renin activity after renal ischemia and hemorrhage. *Kidney International*, **7**, 331–341.

WEINBERGER, M.H., AOI, W. & HENRY, D.P. (1975). Direct effect of beta-adrenergic stimulation on renin release by the rat kidney slice in vitro. *Circulation Res.*, **37**, 318–324.

WHORTON, A.R., MISONO, K., HOLLIFIELD, J., FROLICH, J.C., INAGAMI, T. & OATES, J.A. (1977). Prostaglandins and renin release: stimulation of renin release from rabbit renal cortical slices by PGI_2. *Prostaglandins*, **14**, 1095–1104.

WONG, P. Y-K, LEE, W.H., CHAO, P. H-W, REISS, R.F. & MCGIFF, J.C. (1980a). Metabolism of prostacyclin by 9-hydroxyprostaglandin dehydrogenase in human platelets. *J. biol. Chem.*, **255**, 9021–9024.

WONG, P. Y-K, MALIK, K.U., DESIDERIO, D.M., MCGIFF, J.C. & SUN, F.F. (1980). Hepatic metabolism of prostacyclin (PGI_2) in the rabbit: Formation of a potent novel inhibitor of platelet aggregation. *Biochem. biophys. Res. Comm.*, **93**, 486–494.

WONG, P. Y-K, MCGIFF, J.C., SUN, F.F. & LEE, W.H. (1979). 6-Keto-prostaglandin E_1 inhibits the aggregation of human platelets. *Eur. J. Pharmac.*, **60**, 245–248.

YUAN, B., TAI, C.L. & TAI, H-H (1980). 9-hydroxyprostaglandin dehydrogenase from rat kidney. *J. biol. Chem.*, **255**, 7439–7443.

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