RENAL CONCENTRATIONS OF PROSTAGLANDIN E IN ACUTE AND CHRONIC RENAL ISCHEMIA*

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SUMMARY: We have applied a radioimmunoassay for prostaglandins to the measurement of PGE in renal tissue and serum in models of renal hypertension in rats. Chronic renal-induced hypertension causes increased concentrations of PGE in the ischemic kidney and elevated serum levels of PGE. Acute renal clamp models also result in increased PGE concentrations in the clamped kidney, but inhibition of prostaglandin synthesis by indomethacin treatment does not influence the frequency of hypertensive responses following release of the clamps.

Prostaglandins of the E group are normally synthesized in high concentrations in renal medulla[1,2]. Pharmacologic studies have disclosed that PGE compounds, like PGA's, cause renal vasodilitation, increase effective renal plasma flow, stimulate natriuresis, and as a result, lower blood pressure[3,4,5]. Several investigators have proposed that these actions serve to protect the kidney against any injurious event which induces relative renal ischemia[6]. This concept has been substantiated to a degree in studies with angiotensin. Infusion of angiotensin into the renal artery releases large quantities of prostaglandin-like material into the renal vein. After treatment of dogs with indomethacin, a potent and specific inhibitor of prostaglandin synthesis[7,8,9], angiotensin II causes a greater decrease in renal blood flow than before indomethacin treatment[10], suggesting that prostaglandins blunt the angiotensin vasoconstrictor effect. There are also indications that renal ischemia alone leads to release of prostaglandin-like substances[11] (measured by superfused organ bioassay[12,13]). Renal prostaglandin-E-like lipids appear to increase in induced renal hypertension in rabbits[14,15,16]. In two human experiments, "renal vasodepressor lipid" was found in higher concentrations in the ischemic than in the adequately "perfused" portion of the renal medulla[17] and was also found in high concentrations in the

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renal veins of patients with renal hypertension[18]. While these data appear to implicate prostaglandins in the protection and maintenance of renal blood flow, the experiments described above measure prostaglandin-like activity using biological assay systems.

We have recently described a radioimmunoassay system capable of accurately and specifically measuring concentrations of PGE in plasma and tissue[19,20] We have utilized this radioimmunoassay system to evaluate the role of PGE in renal ischemia and renal-induced hypertension by measuring concentrations of PGE in rat kidney and serum after preparation of experimental models of acute and chronic renal ischemia.

METHODS: Chronic Experiments - Six male 200 Gm rats were anesthetized with pentobarbital anesthesia. Through a midline laparotomy, three or four arterial branches to the lower pole of the left kidney were ligated, causing immediate blanching of the ischemic area of the kidney. The incisions were closed and the animals allowed to recover from anesthesia. Four weeks later the rats were weighed, and under urethane anesthesia (1.2 g/kg), their blood pressures were measured, (P-1000 linear-core transducer-Physiograph). After recording the blood pressures, the animals were exsanguinated either via the carotid arterial cannula or by decapitation. The midline incisions were opened and both kidneys removed immediately and weighed. Each kidney was then immediately homogenized into organic solvent (see below). Serum samples were handled identically. Five normal rats were similarly subjected to the same collection of serum and kidney samples.

Acute Experiments - We have also utilized acute renal pedicle clamping as an acute renal hypertensive model[21]. Upon release of the clamp after 4-1/2 hours of ischemia, renal release of renin results in hypertension in 40% of rats. If in the non-responding rats, prostaglandins prevent the hypertensive response, inhibition of prostaglandin synthesis should result in a consistent hypertensive response to release of the renal clamp. We have thus performed 4-1/2 hour renal clamp experiments in 10 control rats and 10 rats

treated with 10 mg/kg indomethacin intraperitoneally, the evening before and the morning of the experiments. Under urethane anesthesia, 1.0 - 1.2 g/kg, left renal pedicles were cross-clamped and the animals treated with phenoxybenzamine 20 mg/kg intraperitoneally. Four hours later the animals received propanalol, 12 mg/kg, (propanalol and phenoxybenzamine block the effects of catecholamines released by angiotensin) and 15 minutes later the clamps were released and blood pressure monitored by transducers connected to carotid artery cannulas. Five minutes thereafter, the animals were exsanguinated and the kidneys treated exactly as in the chronic experiments.

Measurement of PGE - Kidney samples were homogenized immediately in 3 ml of an organic solvent system consisting of ethyl acetate: isopropanol: 0.1 N HCl, 3:3:1, apparent pH 6.0. After formation of two phases by the addition of two volumes of ethyl acetate and three volumes of water, the organic phase was removed and dried at 55°C in air. The samples were then separated into three major prostaglandin groups by silicic acid chromatography; the PGE-containing fraction was developed using benzene, ethyl acetate: methanol, 60:40:2[20]. Serum samples were processed identically; recovery of [^{3}H] PGE_1 from serum or plasma averages 70% with this technique. PGE concentrations in the appropriate silicic acid fractions from renal tissue and serum were measured by radioimmunoassay using [^{3}H]PGE, and antibody elicited by immunization of rabbits with PGE_1 conjugated to keyhole limpet hemocyanin[19]. Antibody-bound [^{3}H] PGE_1 was separated from free label using dextran-coated charcoal[22]. Sensitivity of the assay system is 5 pg of PGE_1 and specificity for PGE_1 is achieved both by measuring fractions containing only PGE and by using antibodies with specificity for PGE_1[19]. (PGE_2 cross-reacts best, 12%).

RESULTS: Chronic Experiments - The chronic renal ischemic model consistently resulted in moderate sustained hypertension; at 4 weeks, mean blood pressures ranged from 115 to 188 mm Hg and averaged 154 mm Hg (Table 1). In each of the ischemic kidneys, the lower poles (both medulla and cortex) were firm and shrunken; production of renal ischemia resulted in an average

TABLE 1. CHRONIC EXPERIMENTS

	Animal Weight (g)	Average Blood Pressure	PGE Concentrations			Renal Weights	
			Serum (pg/ml)	Left Kidney (pg/g)	Right Kidney (pg/g)	Left (g)	Right (g)
Renal Hypertensive							
1.	233	188	-	2740	2290	1.05	1.44
2.	238	155	1300	1600	1050	0.97	1.02
3.	266	155	2110	1190	500	1.06	1.24
4.	267	135	952	1530	970	1.02	1.16
5.	266	115	1088	1460	650	0.66	1.53
6.	239	175	2462	2250	1770	0.54	1.18
Mean ± S.E.M.	252 ± 7	154 ± 10	1583 ± 298	1962 ± 383	1205 ± 282	0.88 ± 0.09	1.26 ± 0.08
Normal							
1.	250	110	606	583	882	0.97	0.99
2.	257	100	1803	2260	2060	0.97	0.99
3.	260	90	673	3340	1763	1.00	1.00
4.	290	100	485	647	770	1.72	1.64
5.	290	105	343	374	1258	1.64	1.55
Mean ± S.E.M.	269 ± 9	100 ± 4	737 ± 267	1440 ± 582	1347 ± 249	1.12 ± 0.29	1.07 ± 0.27

30% decrease in renal weight as compared with the contralateral control kidneys. Serum concentrations of PGE (corrected for 30% losses during extraction using [^{3}H] PGE_1 tracer) were consistently and significantly ($p<.05$) higher in hypertensive rats compared to serum levels in unoperated controls. Using this radioimmunoassay, plasma (plasma and serum give identical values) PGE_1 levels in 40 normal humans averaged 385 $\pm$ 30 pg/ml and ranged from 139 to 1011 pg/ml; among the control group, 4 of 5 rats had normal levels of PGE whereas 4 of the 5 hypertensive rats had elevated PGE levels. Elevation of serum PGE concentrations reflected increases of renal concentrations of PGE. Although there was some variation in renal PGE concentrations among the control kidneys, PGE concentrations in right and left kidneys from unoperated controls averaged 1347 and 1440 pg/g respectively. Among the hypertensive rats, PGE concentrations in contralateral control kidneys, 1205 pg/g, were similar to those in the normotensive rats. On the other hand, ischemic kidneys contained much more PGE, 1962 pg/g, than the controls. Using statistical analysis for paired data, this difference was statistically significant ($p<.02$).

Acute Experiments - In contrast to the chronic renal hypertensive model, acute production of renal ischemia by clamping the renal pedicle resulted in an average of 53% increase in renal weight, compared to unclamped contralateral control kidneys. Because of considerable variation in renal PGE concentrations, differences between ischemic and control kidneys were not statistically significant. However, the ischemic kidneys contained an average of 3.1 times as much PGE as did the contralateral normal kidneys. Indomethacin treatment did not result in any change in the frequency of hypertensive response to clamp release, (2 of 10 in treated and in untreated rats) despite the fact that indomethacin reduced prostaglandin concentrations in serum and in both clamped and unclamped kidneys an average of 86.7% (only 2/10 serum and 3/20 renal samples had any measureable PGE), indicating that effective pharmacologic concentrations had been obtained.

DISCUSSION: On the basis of the above results, it is clear that PGE

release is altered in association with changes in renal blood flow. The kidney appears to respond to ischemia by synthesizing larger amounts of prostaglandins. PGE is very rapidly inactivated by oxidation of the 15 hydroxyl group to a keto-derivative[23,24], predominantly in the lungs[25-27]; 15 keto prostaglandins do not cross-react in the radioimmunoassay system[28]. Other prostaglandin metabolites could possibly cross-react with anti-PGE_1, but although this might alter absolute concentrations of PGE_1, it should not affect the difference between normal controls and renal hypertensives. Another factor that must be considered is arachidonic and which is present in microgram concentrations per gram of renal tissue. Since arachidonic acid is so poorly extracted by the extraction-chromatography method ($< 3\%$ recovered) and cross-reacts so poorly with anti-PGE_1 (less than 10^{-5}), even at those high concentrations, arachidonic acid should not interfere with the measurement of PGE. The ability of indomethacin to abolish renal PGE concentrations also attests to the lack of interference by arachidonic acid. Thus, the elevation in renal prostaglandin concentrations and particularly the doubling of the serum PGE concentrations reflect an enormous continuous output of PGE in response to decreased renal perfusion. Despite this, liberation of prostaglandin is apparently not sufficient to counteract the renal response to angiotensin. In the chronic experiments, renal hypertension persisted despite elevation of PGE release and in the acute experiments, abolition of prostaglandin synthesis did not alter the rate of hypertensive response to clamp release.

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