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Enhanced Erythropoietin and Prostaglandin E Production in the Dog following Renal Artery Constriction[1] (39244)

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The precise mechanism of erythropoietin (ESF) production and release from the kidney still remains obscure although it is well known that many forms of hypoxia, e.g., anemic, hypoxic, ischemic, and histotoxic, are capable of increasing erythropoietin production by the kidney (1). Recent observations (2, 3) indicate that following renal artery constriction in the dog, there is a release of prostaglandins (PG) into the renal venous blood. We have reported recently (4–6) that both hypoxic and ischemic hypoxia are capable of producing a significant increase in prostaglandin E (PGE) release into the renal venous effluent, which was blocked by the potent prostaglandin synthetase inhibitor, indomethacin. We have also demonstrated (7, 8) that indomethacin is capable of blocking erythropoietin production as well.

Thus, the present studies were carried out to assess the relationship between PGE release and erythropoietin production by the kidney during a 12-hr ischemic hypoxic stimulus induced by means of renal artery constriction as well as the effects of indomethacin on these changes.

Materials and methods. Twelve female mongrel dogs (six control and six indomethacin-treated) weighing 14.5–21 kg were used in this study. The animals were anesthetized with sodium pentobarbital (30 mg/kg iv), and the left femoral arteries were cannulated for monitoring systemic arterial blood pressure (Statham P23AC pressure transducer) and the collection of arterial blood samples for erythropoietin bioassay. The animals were subjected to retroperitoneal laparotomies permitting extirpation of the right kidney and the exposure of the left kidney through a flank incision. A branch of the left ovarian vein was isolated and a cannula was inserted through this vein into the left renal vein to collect renal venous blood samples for PGE assay. Utilizing a square-wave electromagnetic blood flowmeter (Carolina Medical Electronics, Model 501) and either an 8- or 10-mm circumference flow probe, renal blood flow (RBF) was monitored with the probe placed around the left renal artery. Systemic arterial blood pressure (BP) and RBF were recorded continuously throughout the experimental period on a Grass oscillographic recorder (Model 7PCPA).

After the initial zero time parameters (BP, RBF) and blood samples for PGE and erythropoietin were collected, a vascular occluder (Model OC12, IN VIVO Metric Systems) was placed around the left renal artery between the flow probe and the kidney. Renal blood flow was reduced to 30% of normal and sustained throughout the 12-hr experimental period with minor adjustments as needed. Six of the 12 dogs in the experimental group were pretreated with indomethacin (5 mg/kg orally) 18 and 2 hr prior to the application of the vascular occluder. Anesthesia was maintained throughout the experiments by administration of sodium pentobarbital as required via a cannula in the femoral vein.

Blood samples for erythropoietin and

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PGE assay were withdrawn at zero time prior to renal artery constriction and at 1, 4, 8, and 12 hr postconstriction. Samples for erythropoietin assay were centrifuged, and the plasma separated and frozen at −80° until assayed. Erythropoietin was measured utilizing a modification of the exhypoxic polycythemic mouse bioassay of Cotes and Bangham (9), the details of which we have previously reported (7). Erythropoietin titers were expressed as the 48-hr percentage of ^{59}Fe incorporation into red blood cells of polycythemic mice when compared with a 3 point International Reference Standard (IRP-II) erythropoietin dose-response regression line. Blood samples for PGE analysis were allowed to clot and the serum was separated by centrifugation, frozen with liquid nitrogen, and lyophilized prior to storage at −80°. PGE levels were determined by radioimmunoassay utilizing the technique of Jubiz *et al.*, as previously described (10).

Results. The changes in mean arterial blood pressure and renal blood flow (mean ± SE) during 12 hr of renal artery constriction (RAC) are presented in Fig. 1. It can be seen that RAC to 30% of normal in both the control and indomethacin-treated groups produced a rapid though not significant rise ($P < 0.2$) in blood pressure which was sustained above zero-hour levels throughout the experimental period, especially during the first 4 hr. The reduction in renal blood flow was well maintained during the entire experimental period in both groups of dogs.

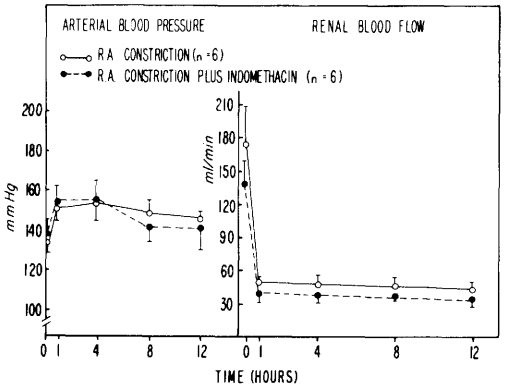

FIG. 1. Arterial blood pressure and renal blood flow in dogs following renal artery constriction and indomethacin treatment. Values are the mean ± 1 SE.

Figure 2 shows the changes in PGE levels in the renal venous effluent and in erythropoietin (ESF) titers during the course of the 12-hr renal artery constriction. RAC produced a significant elevation in erythropoietin titers ($P < 0.05$) at 8 and 12 hr as compared to the levels of the indomethacin-treated group. This elevation in plasma erythropoietin levels following RAC was accompanied by a concomitant increase in PGE levels in renal venous effluents. This figure also illustrates the *in vivo* effects of indomethacin on PGE and erythropoietin. While RAC produced an elevation in PGE levels in the control group, pretreatment with indomethacin significantly ($0.1 > P > 0.05$) blocked this increase. A similar effect was seen with regard to erythropoietin titers which, though elevated by RAC in the control group, were not significantly increased above zero-time levels in the group pretreated with indomethacin.

Discussion. The kidney has been shown to be the site of release of the prostaglandins E_2 and $F_{2\alpha}$ (11). It has also been suggested that the prostaglandins may enhance erythropoiesis by a direct action upon the bone marrow (12) as well as acting upon the kidney to increase erythropoietin production (13, 14). Paulo *et al.* (14) and Gross *et al.* (15) have shown with the isolated, programmed hypoxic dog kidney perfused with PGE_1 and PGE_2, respectively, an increase in erythropoietin titers in the perfusate during 5 hr of perfusion. Paulo *et al.* (14) also observed significantly increased levels of renal adenosine 3′,5′-monophosphate (cAMP) in the kidneys perfused with PGE_1 as compared to saline controls.

Although the renal prostaglandins (16) are probably synthesized continuously, with the exception of PGA_2, it is unlikely that the PGs can act as circulating hormones because of their instability in blood and other tissue fluids as well as their target specificity. PGE_2 is the major renal prostaglandin (17) and is essential for the regulation of blood distribution between the kidney cortex and medulla and for the maintenance of resting blood flow. The finding that the prostaglandins are synthesized primarily in the medulla, whereas erythropoietin synthesis probably occurs at cortical sites (1) poses an interesting problem. Since both PGE_2 and

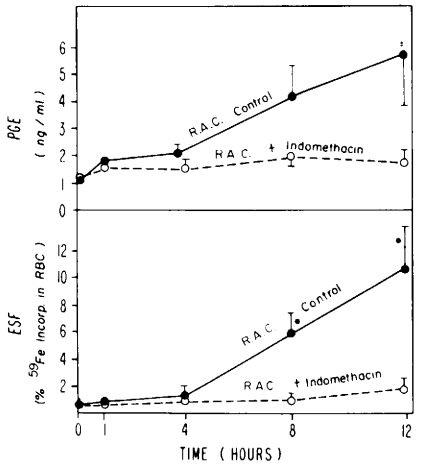

FIG. 2. The effects of indomethacin on serum prostaglandin E (PGE) levels and plasma erythropoietin (ESF) titers of dogs (n = 6 in each group) following renal artery constriction (RAC) to 30% of normal flow. Values are the mean ± 1 SE. ‡, Significantly different from RAC + I ($0.1 > P > 0.05$). *, Significantly different from RAC + I ($P < 0.05$).

$PGF_{2\alpha}$ have been identified in the urine (18) it is quite likely that the medullary renal prostaglandins may affect cortical structures after they have traversed the ascending limb of the loop of Henle to reach the cortex via the distal tubule.

In the present study, we have found that renal artery constriction to 30% of normal flow produced a significant increase in plasma titers of erythropoietin and PGE levels in the renal venous effluent. In addition, indomethacin was found to block the increase in erythropoietin titers and PGE levels. Lonigro *et al.* (16) have demonstrated that indomethacin inhibited prostaglandin E synthesis and, hence, release while reducing renal blood flow. More recently, Kirschenbaum and coworkers (19) have shown that indomethacin and melcofenamate, both inhibitors of PG synthesis though structurally unrelated, decrease total renal blood flow in association with a redistribution of blood flow to the outer cortical nephrons. It has also been noted that indomethacin acts as a prostaglandin synthetase inhibitor at a step prior to the formation of an endoperoxide intermediate, depressing the formation of PGE while having no specific hemodynamic effects of its own. Thus, decreased levels of PGE are a direct consequence of the inhibition of PGE biosynthesis by indomethacin.

In addition to the effects of prostaglandins on cortical and medullary hemodynamics, it has also been observed that the actions of PGE_2 are largely expressed by the regulation of intracellular levels of cAMP (20). Kuehl and coworkers (21) have postulated that prostaglandins act as modulators interacting with a membrane-bound adenylate cyclase leading to increased cAMP formation. Rodgers, Fisher, and George (22) have convincingly demonstrated a role for cAMP and adenylate cyclase in the mechanism of the renal release of erythropoietin following a variety of erythropoietic stimuli. Furthermore, we have reported that PGE_2 (15) produces a dose-dependent increase in ^{59}Fe incorporation in red blood cells of exhypoxic polycythemic mice in addition to the stimulatory effects on erythropoietin production by the isolated, perfused dog kidney as noted previously (14, 15). Thus with the data presented here it would follow that inhibition of prostaglandin E synthesis might affect erythropoietin production via adenylate cyclase, thus decreasing renal cortical cAMP levels and hence erythropoietin release.

Summary. Renal artery constriction (RAC) to 30% of normal flow for 12 hr in the unilaterally nephrectomized dog produced a marked increase in both erythropoietin titers and prostaglandin E (PGE) levels in the blood. In dogs pretreated prior to RAC with indomethacin, a potent inhibitor of prostaglandin synthetase, there was no significant increase in either PGE or erythropoietin levels as compared to zero-time control values. These data suggested an involvement of renal PGE in the generation of erythropoietin following RAC.

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