Alterations in the fraction of oxygen in inspired gas affect rates of renal prostaglandin E_2 synthesis in anesthetized dogs[1]

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Summary. Reductions of oxygen in inspired gas from 20% to 15%, in anesthetized dogs, reduced arterial PO_2 and increased the renal efflux of PGE_2 but not $PGF_{2\alpha}$. Renal blood flow, blood pressure, plasma renin activity as well as arterial pH and PCO_2 were unaffected. PGs may mediate the renal hemodynamic or excretory consequences of alterations in PO_2. In addition, minor variations in PO_2 might account, in part, for the variable renal venous PGE_2 concentrations reported under basal conditions.

The prostaglandins (PGs), a group of highly biologically active unsaturated lipids, have been implicated as participants in the local adjustments to blood flow which occur in response to changes in oxygen delivery. Thus, not only was PG concentration in venous effluent reported to be increased following periods of oxygen deprivation in several vascular beds[2-5] but, in addition, hemodynamic responses to changes in oxygen availability were reported to be abolished when PG synthesis was inhibited[4-7]. In the kidney, for example, Herbaczynska-Cedro and Vane reported that reactive hyperemia was eliminated by pretreatment with indomethacin, a potent inhibitor of PG synthesis[7]. In addition, Millard et al. have presented evidence suggesting that increased renal synthesis of PGs protects the renal circulation of the fetal lamb in utero against excessive vasoconstriction consequent to severe hypoxemia[8]. The physiologic significance, however, of studies in which increased rates of PG synthesis occur consequent to either anoxia or extreme hypoxia has been questioned by Block et al.[9]. These investigators suggest that, under conditions of severe oxygen deprivation, increased PGs occur most likely because of the trauma imposed by the stimulus, and that PGs so derived do not function as physiologic modulators of vascular resistance. The aim of the present study was to evaluate whether mild degrees of hypoxemia could influence rates of renal PG synthesis.

Methods. Morphine (2 mg/kg, s.c.) and chloralose (100 mg/kg, i.v.) anesthetized, male mongrel dogs (26–32 kg), fasted overnight but allowed water ad libitum, were ventilated with a cuffed endotracheal tube attached to a Harvard Ventilator (model 618). The abdomen was opened by a transverse incision and 1 renal artery fitted with an electromagnetic flow-probe (inside diameter 3.0–4.0 mm) attached to a Statham flow-meter (model SP2202). The ipsilateral renal vein was catheterized via a femoral vein to obtain samples for PG determination. Arterial samples for PG concentrations, plasma renin activity (PRA) and blood gas analyses were obtained from an aortic catheter introduced via the brachial artery. Mean arterial blood pressure was measured with a Statham pressure transducer (model P23ID) attached to an aortic catheter introduced through a femoral artery. With the ventilator open to room air, tidal volume and rate were adjusted so that control values for arterial PCO_2, PO_2 and pH were within the range of values considered normal for unanesthetized dogs[10]. Neither tidal volume nor ventilatory rate were altered thereafter. The fraction of oxygen in the inspired gas was varied by attaching balloons containing the desired oxygen concentration in nitrogen to the ventilator. Renal venous and aortic samples for the radioimmunoassay[11] of PGE_2 and $PGF_{2\alpha}$ were obtained simultaneously during the control period and 2 min after altering the fraction of oxygen in the inspired gas. Blood samples (35 ml) for assay were collected in syringes containing EDTA (35 mg) and transferred immediately to chilled tubes containing meclofenamate (175 µg). After centrifugation, ^{3}H-PGE_2 and ^{3}H-$PGF_{2\alpha}$ (1.6 nCi of each) were added to the plasma to permit estimates of losses incurred on extraction and purification. A 10-ml aliquot, acidified to pH 3 with 2 M citric acid, was extracted (×2) with cyclohexane-ethyl acetate (1:1 by vol.). Following reduction of the organic phase by evaporation in vacuo, PGs were separated on silicic acid columns using solvent mixtures (benzene:ethyl acetate:methanol) of increasing polarity. The PGE and the PGF fractions were evaporated to dryness under nitrogen at 40 °C and brought up in 0.8 ml buffer. For radioimmunoassay, performed in duplicate for each sample, the PG fraction (0.1 ml) and the PG antiserum (0.1 ml) were incubated with a fixed quantity of ^{3}H-PGE_2 or ^{3}H-$PGF_{2\alpha}$ (sp. act. 130 Ci/mM). Bound was separated from free PG by the addition of a dextran-charcoal solution. Standard curves were generated for each assay. Concentrations of PGE_2 and $PGF_{2\alpha}$, corrected for losses, are expressed as pg/ml blood. For PRA, blood (5 ml) was collected in chilled tubes containing EDTA (5 mg) and centrifuged. The plasma was removed, the pH reduced to 6.0 and the amount of angiotensin I generated during a 1-h incubation period determined. Significant differences between control values and those of the experimental period were determined by the Student's t-test. P-values of 0.05 or less were considered statistically significant.

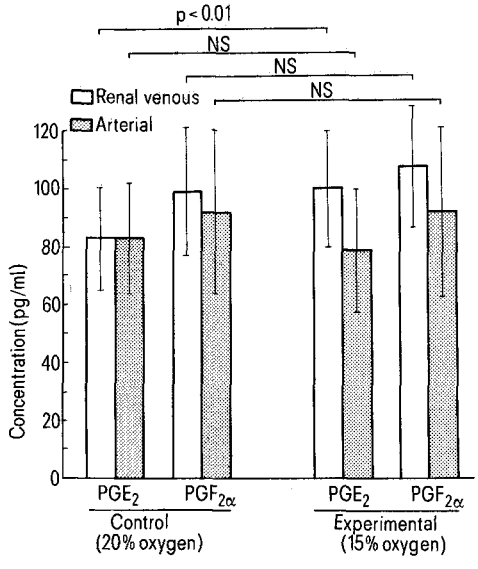
Effects on blood gas composition, arterial pH, plasma renin activity, renal blood flow and blood pressure in anesthetized dogs of reducing the fraction of oxygen in the inspired gas from 20% to 15%

	PO_2 (mm Hg)[a]	PCO_2 (mm Hg)[a]	pH (units)[a]	Plasma renin activity (ng/ml/h)[a]	Renal blood flow (ml/min)[a]	Mean arterial blood pressure (mm Hg)[a]
Control	113±4	31±1	7.360±0.009	4.6±2.0	222±24	114±6
Experimental	88±3	30±1	7.362±0.008	5.0±2.0	222±24	116±4
Probability[b]	<0.001	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]

[a] Values are means ± SEM for 9 experiments in 9 dogs. During the control periods, the animals were ventilated with room air; during the experimental period, the animals were ventilated with 15% oxygen in nitrogen. [b] Statistical analyses were made, using the paired t-test, for experimental vs control periods. [c] NS indicates $p > 0.05$.

Results. In 9 dogs, a reduction in the fraction of oxygen in the inspired gas from 20% to 15% was associated with a 24±2 mm Hg decrease in the oxygen tension of arterial blood within 2 min (table). Simultaneously, the concentration of PGE_2 in renal venous blood increased significantly ($p < 0.01$); whereas, arterial concentrations of PGE_2 were not affected (fig.). In contrast, neither arterial nor renal venous concentrations of $PGF_{2\alpha}$ were affected by this maneuver (fig.). Similarly, this reduction of the fraction of oxygen in the inspired gas did not affect PCO_2, pH, PRA, renal blood flow or mean arterial blood pressure (table). In 4 of the 9 dogs the fraction of oxygen in the inspired gas was further reduced from 15% to 5% (not shown). This produced an additional 114±48% increase in the renal venous concentration of PGE_2; however, this latter maneuver was associated with a 30±2% decrease in renal blood flow so that the renal PGE_2 efflux (product of renal blood flow and renal venous concentration) decreased by 59±9%. To exclude the possibility of time-dependent changes in renal venous prostaglandin concentrations, identical procedures were carried out in 4 additional experiments except that the animals were not subjected to a reduced fraction of oxygen in the inspired gas. 2 and 40 min after the sham reduction of the fraction of oxygen in the inspired gas, the mean (±SEM) differences from control concentrations for PGE_2 in renal venous blood were 0.2±4.0 and −2.6±7.0 pg/ml, respectively. Therefore, the increase in renal venous concentration of PGE_2 does not appear to be a time-related event.

Discussion. The requirement for oxygen in the biosynthesis of PGs has been recognized for a decade and a half[12,13]. From a physiological standpoint, however, the relationship between oxygen supply and rates of PG synthesis is less clear[9,14]. In skeletal muscle, heart and kidney, for example, increased rates of PG synthesis have been suggested to occur in the presence of a reduced oxygen supply. In view of the fact that increased rates of PG synthesis have been observed or inferred only during extreme hypoxia[8] or after periods of anoxia[2-7], questions have been raised regarding physiological significance of the observations[9,14]. In the work presented here, a small reduction in the fraction of oxygen in the inspired gas produced a small, but significant,



Effects of reducing the fraction of oxygen in the inspired gas from 20% (left, control) to 15% (right, experimental) on renal venous and arterial concentrations of PGE_2 and $PGF_{2\alpha}$ in chloralose-anesthetized dogs. Statistical significance was determined using the paired t-test. NS indicates no statistical significance.

increase in the rate of PGE_2 synthesis within the kidney. This increase was not time-related nor was it related to change in PRA. Whether the mechanism of increased rates of renal prostaglandin synthesis was indirect, i.e., mediated by nervous or other hormonal systems, or the result of a direct effect of reduced PO_2 on prostaglandin synthesizing mechanisms is not revealed by the present work. In addition, definition of the mechanism(s) which would favor synthesis of PGE_2 over $PGF_{2\alpha}$ was not addressed, but may eventually be resolved in terms of suppression of 9-keto-reductase activity, an enzyme which reduces PGE_2 to $PGF_{2\alpha}$[15]. Alternatively, hypoxia may have affected those enzymes which regulate the formation of PGE_2 and $PGF_{2\alpha}$ from endoperoxide intermediates[16]. The mechanism(s) notwithstanding, enhanced PGE_2 synthesis may account for the increases in urine flow observed under conditions of mild hypoxia[17], perhaps related to PGE_2 inhibition of the actions of antidiuretic hormone[18]. In the present study, when more severe hypoxia was produced, the efflux of renal PGE_2 decreased. The reduction in the efflux of PGE_2 might have been related either to a general failure of PG synthesis within the kidney because of insufficient oxygen or to a shift in synthesis away from PGE_2 to some other product of cyclooxygenase activity; e.g., prostacyclin (PGI_2). The latter consideration, if true, would help to explain the results of Millard et al.[8] in which prostaglandins were implicated in the control of the renal circulation under conditions of rather severe hypoxia.

Speculation aside, the present results are important from 2 standpoints: a) they demonstrate that mild degrees of hypoxia can stimulate prostaglandin synthesis; b) they suggest that minor variations in alveolar and/or arterial oxygen tension might contribute to the highly variable concentrations of PGE_2 reported to occur in renal venous blood under basal conditions.

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