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Indomethacin Blockade of Albuterol-Induced Erythropoietin Production in Isolated Perfused Dog Kidneys (40619)[1]

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The formation and/or release of erythropoietin (Ep) by the kidney have recently been demonstrated to be under the control of both hormonal (1–4) and neural (3, 5, 6) influences. Thus, Ep production following exposure to hypoxia has been found to be abolished by cholinergic (6) and β-adrenergic (3) blockade or renal denervation (3). In addition, our laboratory has previously reported that activation of the β-adrenergic nervous system increases Ep production in normoxic animals (7). Albuterol, a specific β-2 adrenergic agonist (8), has been found to produce a significant increase in serum Ep titers when administered to rabbits (7, 9). This effect was abolished by the simultaneous administration of a β-2 adrenergic blocking agent (9). Thus, it seems clear from these *in vivo* studies that the effect of albuterol on Ep production is mediated by β-2 adrenergic receptors. On the other hand, the role of prostaglandins inhibition on the direct effects of β-2 adrenergic agonists on Ep elaboration in renal cells has not heretofore been investigated.

Therefore, we have undertaken studies of the effects of albuterol on Ep production in programmed isolated posthypoxic dog kidneys during normoxemic and hypoxemic perfusion. In addition, experiments were carried out to determine if the postulated β-2 adrenergic activation of Ep production requires intrarenal synthesis of renal prostaglandins, as has been shown for the elaboration of Ep during hypoxia (2), by studying the effects of indomethacin, an inhibitor of prostaglandin synthesis, on albuterol-induced kidney production of Ep.

Materials and methods. Female mongrel dogs (10–25 kg, b.w.) with hematocrits between 35–44% were exposed to hypoxia (0.42 atm) for 2 hr prior to removal of the kidney for perfusion. The left kidney (18–58 g) was removed under sodium pentobarbital (30 mg/kg i.v.) anesthesia via abdominal laparotomy and renal arteries and ureter cannulated with polyethylene tubing. At the same time 500 ml heparinized blood (500 U sodium heparin/kg b.w.) was collected from the carotid artery to be used as the perfusate. Kidneys were flushed with a single pass of 500 ml heparinized (0.5 U/ml) lactated Ringer's solution at 37° to remove the blood contained in the kidney. Thereafter, the kidneys were placed in a closed-circuit perfusion system consisting of an organ-warming chamber, membrane blood oxygenator, blood reservoir, heating coil, and perfusion pump as described previously (10). Isolated kidneys were perfused for 5 hr at 37° at a flow rate of 3 ml/g/min with 500 ml recirculating blood (adjusted to a hematocrit of 35%). Urine was returned to the perfusion system. Blood gases were analyzed (Instrumentation Labs Model 127 blood gas analyzer) initially and at hourly intervals. Blood pO_2 and pCO_2 were kept constant by equilibration with N_2, CO_2, and O_2 in a sialastic tubing oxygenator (arterial pCO_2 35–45 mmHg, pH 7.35–7.45). Arterial pO_2 was maintained between 60–80 mm Hg in normoxemic perfusions and between 18–25 mm Hg in hypoxemic perfusions. Perfusion pressures (Statham pressure transducer P23AC) and perfusate temperature were monitored continuously. The following substances were regularly added to the perfusion fluid: 500,000 U of penicillin G at the beginning of the experiment and subsequently 100,000 U at 2 and 4 hr of perfusion; 2000 U sodium heparin at 2 and 4 hr; and 75 mg glucose hourly. Albuterol (500 μg/liter blood, Schering Inc., N.J.) dissolved in saline or saline alone (50 μl) was added to the blood prior to the perfusion. Blood samples (20 ml) for the determination of plasma erythropoi-

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etin titers were removed from the perfusate after 5 min (0-hr value), 3 and 5 hr of perfusion.

In the experiments where indomethacin was used the dogs were pretreated with the drug (5 mg/kg p.o.) 16 hr and 15 min before exposure to hypoxia. The erythropoietic activity of the plasma samples from the perfusion system was determined using the exhypoxic polycythemic mouse bioassay for Ep (11). HAM/JCR strain female mice were made polycythemic by exposure to hypoxia for 2 weeks (0.42 atm for 22 hr/day). Groups of five mice were injected s.c. with either saline, human urinary Ep standardized against the International Reference Preparation, or 0.5 ml plasma on Days 6 and 7 after their removal from the hypobaric chamber. On Day 8 the animals received 0.5 μCi ^{59}Fe citrate i.v. Blood was removed via cardiac puncture on Day 10 and erythropoietic activity of the sample was calculated from the mean 48-hr percentage ^{59}Fe incorporation into red blood cells for each of the mice. Experiments in which the initial erythropoietic activity (0-hr value) of perfusate samples exceeded 5% ^{59}Fe incorporation in the mouse bioassay were excluded from the results (4 out of 34 experiments).

In order to prove that the erythropoietic activity of perfusate samples was due to erythropoietin and not to possible effects of the drug remaining in the plasma samples being assayed on bone marrow progenitor cells (9), the effect of antierythropoietin immune serum (anti-Ep) on the erythropoietic activity of plasma samples was assessed. Plasma was incubated with rabbit anti-Ep serum or normal rabbit serum for 45 min at 37°, followed by removal of the anti-Ep by an additional incubation with goat anti-rabbit γ-globulin (GARGG) for 45 min at 37°. The plasma was then centrifuged and the supernatant assayed for erythropoietin in the polycythemic mouse bioassay. The Ep assay results are expressed as the mean ± SEM percentage ^{59}Fe incorporation in red cell values. The statistical significance of the data was calculated using Dunnett's test for multiple comparisons with a single control or the Student's *t* test for single comparisons.

Results. Effect of albuterol during normoxemic perfusion. As noted in Fig. 1, no significant erythropoietic activity was found in saline-control perfusates at the beginning of the perfusion period (0-hr value). In addition, no increase in Ep titers was observed during a 5-hr perfusion of the isolated programmed posthypoxic dog kidneys with normoxemic (pO2 60–80 mm Hg) blood. When albuterol (500 μg/liter) was added to the blood prior to the beginning of the perfusion, a significant increase in Ep titers was seen in the perfusates. Plasma Ep titers were significantly (*P*

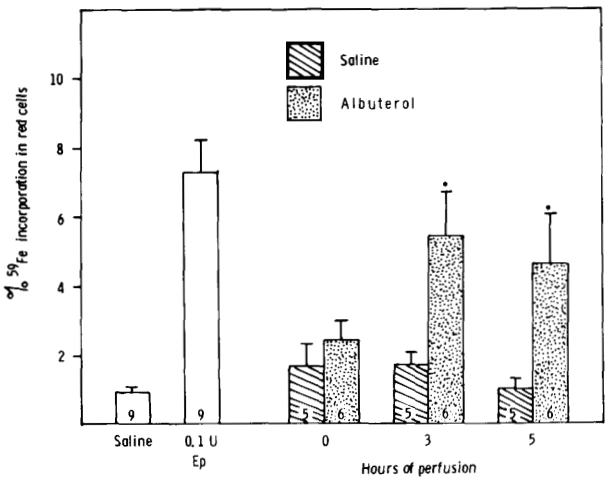

FIG. 1. Effects of albuterol (500 μg/liter) on Ep production in the normoxemic (pO_2 60–80 mm Hg) isolated perfused dog kidney. Each bar is the mean ± SE (numbers at the bottom represent number of experiments). *Significantly ($P < 0.05$) different from saline control value.

< 0.05) higher in albuterol normoxemic than in saline-normoxemic perfused kidney perfusates after 3 and 5 hr.

Effect of albuterol on erythropoietin production during hypoxemic perfusion. The erythropoietic activity in plasma perfusates from saline-hypoxemic (pO_2 18–25 mm Hg) perfused dog kidneys after 3 hr of perfusion (3.34 ± 0.72 percentage ^{59}Fe incorporation in RBC) was significantly ($P < 0.05$) increased above the level detectable in the polycythemic mouse bioassay (0.82 ± 0.14%). As demonstrated in Fig. 2, Ep titers were moderately elevated above their respective 0-hr value after 3 and 5 hr saline-hypoxemic perfusion but the increases were not statistically significant. On the other hand, when albuterol was added to the perfusate a significant increase in Ep titers was found at 3 and 5 hr when compared with that of either saline-hypoxemic perfusate or with the initial 0-hr value.

The possibility that the albuterol in the perfusate and not Ep was responsible for the erythropoietic stimulation in the polycythemic mice was ruled out by incubating the 5-hr albuterol perfusates with either anti-Ep rabbit sera or normal rabbit serum. After the antibody was removed by GARGG, the erythropoietic activity was abolished in the anti-Ep-treated perfusate samples, whereas incubation with normal rabbit serum did not alter Ep titers when assayed in exhypoxic polycythemic mice. These results indicate that the plasma perfusates contained erythropoietin.

Effect of indomethacin pretreatment on albuterol-induced Ep production. As shown in Fig. 3 when dogs were pretreated with indomethacin prior to exposure to hypoxia, no detectable Ep was found during a 5-hr albuterol-normoxemic perfusion. Indomethacin pretreatment caused a decrease in Ep production which was significantly below that seen in nontreated dog kidneys following the addition of albuterol to the perfusate.

Perfusion pressure. In maintaining a constant renal blood flow (3 ml/g/min) during the perfusion some changes occurred in the perfusion pressure. Following an initial autoregulatory decrease in perfusion pressure, a gradual increase in pressure was usually seen over the 5-hr perfusion period. However, no significant differences were found between the treatment groups and Ep production was not correlated with the perfusion pressure in any of our experiments. Mean perfusion pressures at 30 min and 5 hr were 114 and 148 mm Hg without pretreatment of dogs and 119 and 191 mm Hg, respectively, following indomethacin pretreatment.

Discussion. We have previously reported that β-2 adrenergic agonists increase plasma Ep titers in normoxic (7, 9) and hypoxic (12) animals. The present study was undertaken to determine whether this effect is due to a direct renal cell adrenergic receptor activa-

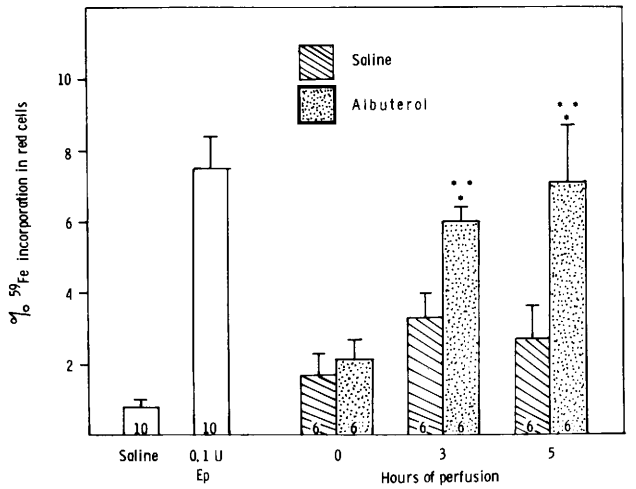

FIG. 2. Effects of albuterol on Ep production in the hypoxemic (pO_2 18–25 mm Hg) isolated perfused dog kidney. Each bar is the mean ± SE (numbers at the bottom represent number of experiments). *Significantly ($P < 0.05$) different from saline control value. **Significantly ($P < 0.05$) different from the respective 0-hr value.

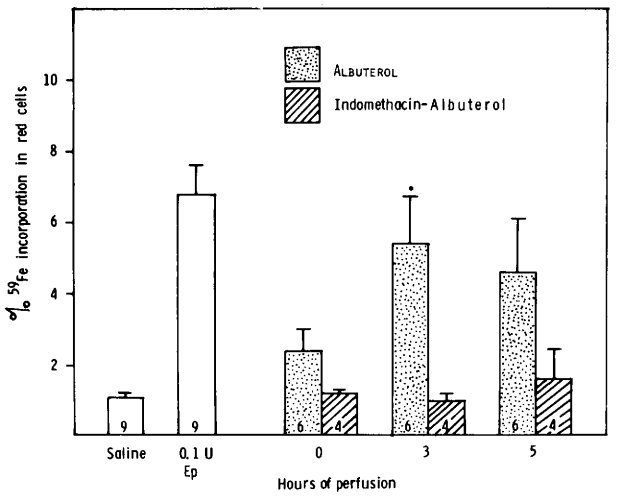

FIG. 3. Effects of pretreatment with indomethacin (5 mg/kg twice) on albuterol-induced Ep production in normoxemic isolated perfused dog kidneys. *Significantly ($P < 0.05$) different from indomethacin pretreatment.

tion of Ep production or to indirect effects due to a β-2 adrenergic activation of prostaglandins release in the kidney.

β-Adrenergic agonists are known to influence respiratory ventilation, blood pressure (8), and renal blood flow (13). Isolated dog kidneys were perfused at a constant flow rate in the present experiments in that the production of Ep is known to be modified by changes in renal blood flow (14). On the other hand, we were unable to detect a correlation between the perfusion pressure and Ep elaboration. In contrast to our earlier studies (10) the current perfusion technique utilized the blood and kidneys from the same dog in the perfusion system. No detectable Ep was found in the blood removed from the 2-hr posthypoxic dogs which was the 0-hr perfusate sample. In addition, no Ep was released from kidneys during the 5-hr perfusion with normoxemic blood [>90% oxy-Hb (15)]. During hypoxemic perfusion ($\triangleq$ 20% oxy-Hb), erythropoietic activity was significantly higher at 3 hr than the ^{59}Fe incorporation values seen in saline-treated polycythemic mice. Addition of albuterol to the perfusate significantly enhanced Ep production in normoxemic and hypoxemic perfused kidneys when compared to saline-control kidney perfusates. The possibility that the erythropoietic activity of the albuterol perfusates was not Ep but caused by direct effects of albuterol on the mouse bone marrow erythroid progenitor cells (9) was ruled out since Ep titers were lower at 0 hr when compared to that of the later time intervals. In addition, erythropoietic activity in the plasma perfusate was also abolished following incubation with anti-Ep.

The significance of β-adrenergic activation in the physiologic control of Ep production is not clear at the present time. Ep is certainly produced at an accelerated rate in adaptation to hypoxia (1). The administration of the β-adrenergic antagonist propranolol has been shown to partially antagonize Ep elaboration during hypoxia (3). In addition, catecholamine levels are known to be increased during hypoxia (16). The concentration of catecholamines in blood of normal dogs is estimated to be 10^{-8} M which is lower than that of the initial concentration of albuterol (10^{-6} M) used in our experiments. However, it is likely that a major part of the β-2 adrenergic agonist is rapidly bound to cell surfaces so that the actual concentration of the free drug at the appropriate receptor site is much lower than can be calculated from the initial concentration. It should be noted that in general marked pharmacological stimulation of Ep release is necessary to produce titers of Ep that can be detected with the biological assay methods currently available which is at best greater than 0.05 U Ep/ml. Considering these data, our results support the hypothesis that the β-adrenergic nervous system plays a physiological role in the regulation of Ep production during adaptation to hypoxia.

It is of interest to consider the possible

mechanisms by which β-2 adrenergic activation increases Ep production in the kidney. β-2 Adrenergic agonists in general have been demonstrated previously to exert their hormonal effects by increasing cell membrane adenylate cyclase activity and thereby result in an increase in the intracellular concentration of cAMP (17). β-Adrenergic agonists are also capable of increasing renal cortical cAMP levels *in vitro* (18). Our earlier work has suggested that the synthesis of Ep is mediated by an increase in renal cAMP levels (19). Therefore, it seems very likely that albuterol increased Ep production by the activation of adenylate cyclase to increase cAMP concentrations in a renocortical cell in the isolated perfused kidney either directly or through prostaglandins release.

In considering the current finding that indomethacin blocked the effects of albuterol on kidney Ep production, evidence has been provided from our laboratory that renal prostaglandins, primarily PGE_2, are involved in the mechanism of kidney elaboration of Ep during hypoxia (2, 10). When dogs were pretreated with the prostaglandin synthetase inhibitor indomethacin prior to exposure to hypoxia, Ep production was abolished during hypoxic stimulation (2). The results presented here indicate that β-2 adrenergic activation of Ep production may also be mediated by a prostaglandin mechanism since indomethacin blocked the enhanced Ep production with albuterol. However, one must consider the possibility that pretreatment of the dogs with indomethacin may also have inhibited the early 2-hr programming phase of the kidney at an early stage of the Ep cascade. Enhanced release of prostaglandins from the kidney has been reported to occur immediately after the beginning of hypoxia (20, 21). We have postulated previously that limited exposure to hypoxia renders the kidney more responsive to erythropoietic stimuli (10), especially since the level of the inactive form of the renal erythropoietic factor (REF, erythrogenin) in the kidney has been shown to increase during hypoxia (19). It is quite possible that indomethacin pretreatment prior to the 2-hr exposure of the dog to hypoxia blocked the synthesis of prostaglandins during the programming phase and prevented the elaboration of REF (erythrogenin) and thereby decreased the effects of albuterol on an early phase of albuterol's effect on Ep production in the isolated perfused kidney. It would be of interest to learn in further studies in the future whether a prostaglandins synthetase inhibitor added directly to the perfusate would interfere with albuterol-induced kidney production of Ep. Our current results do strongly suggest that albuterol exerts its effects, at least in part, by increasing the synthesis of a renal prostaglandin which plays an important role in kidney production and/or release of Ep. Even though β-adrenergic agonists have been implicated in the release of prostaglandins from the kidney (22), further studies on the effects of β-2 adrenergic agonist activation of prostaglandins synthesis and Ep production are necessary to establish the role of renal prostaglandins in β-adrenergic activation of kidney production of Ep.

Summary Albuterol, a specific β-2 adrenergic agonist, was found to produce a significant increase in perfusate levels of Ep in isolated perfused dog kidneys which were programmed by prior exposure to hypoxia (0.42 atm) for 2 hr. Albuterol enhanced Ep titers in perfusates in both normoxemic and hypoxemic perfused kidneys which were significantly higher than that of the Ep level seen in saline controls during a 5-hr perfusion period. The effect of albuterol on Ep production in isolated perfused kidneys was abolished by pretreatment of the dogs with the prostaglandins synthetase inhibitor indomethacin. The inhibition of albuterol-induced Ep elaboration by indomethacin supports our hypothesis that prostaglandins may play a significant role in mediating β-2 adrenergic activation of kidney Ep production.

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