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The Effects of Prostaglandins on Extrarenal Erythropoietin Production (41424)

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Abstract. The E and A series prostaglandins have been reported to stimulate erythropoiesis and renal erythropoietin (Ep) production. In the present study, these prostaglandins also stimulated the elaboration of extrarenal Ep in renoprival animals after hypoxia. This extrarenal response was primarily due to hepatic Ep synthesis; when subtotal hepatectomy (hepx) was followed by nephrectomy, the Ep response to hypoxia was almost completely abolished. The synthetic methylated prostaglandins (16, 16-dimethyl E_2 and (15s)-15 methyl E_2) exerted the most potent effects on both the hepatic and renal Ep response. It is believed that this is attributable, at least in part, to the greater stability of these compounds *in vivo*. Prostaglandins do not appear to be capable of substantially elevating Ep production by the regenerating liver. When compared to vehicle- or saline-injected rats, a greater stimulation of hepatic Ep elaboration after prostaglandin treatment was observed in animals with normal livers than in rats with liver regenerating 72 hr after hepx.

The prostaglandins, a heterogeneous group of compounds formed from unsaturated fatty acids, are diverse in both function and origin (1). Prostaglandins, in addition to their vasoactive properties (2, 3), appear to exert a significant effect on hematopoiesis *in vivo* as well as *in vitro* (4–7). The stimulation of erythropoiesis in plethoric mice caused by treatment with the E series prostaglandins is apparently associated with an elevation in erythropoietin (Ep) levels, since treatment of these animals with anti-Ep immune serum abolished this effect (6). The *in vitro* response of bone marrow cells to Ep was enhanced when the cultures were pretreated with prostaglandin E_1 (4). This potentiation of the Ep effect was also reported in cultures to which prostaglandins E_1, E_2, A_1, A_2, B_1, and B_2 were added (8). Treatment with these compounds resulted in significantly higher BFU-E and CFU-E numbers when compared to controls (8). The prostaglandins act on the cells responsive to Ep (4, 7, 8), but they also stimulate the production of this principle (6, 9) in the intact animal. This increase in Ep production has been attributed to a direct renal action of the E and A series prostaglandins (9) since the kidney is generally acknowledged to be the primary source of this hormone in mammals (10). Although the kidney is the main Ep-producing organ in the adult mammal (10), extrarenal sources of this principle also exist, the most important of which is the liver (11). Substances which stimulate hepatic Ep production may prove useful in treating conditions in which renal elaboration of Ep is low or nonexistent, such as the anemia of chronic renal insufficiency in man. Renocellular damage due to the diseased state of the kidneys may preclude the possibility that kidney Ep production in these patients can be augmented by prostaglandins known to stimulate renal elaboration of this hormone in normal animals. The prostaglandin effect on renal Ep production has been well documented (6, 9) but little information concerning the effects of these cyclic compounds on hepatic Ep has been published (12). The effects of prostaglandins A_1,A_2,E_1,E_2,$F_{2\alpha}$, (15s)-15 methyl E_2, and 16, 16-dimethyl E_2 on hepatic Ep production are reported in the present study.

Materials and Methods. The following prostaglandins were employed in this study: A_1, A_2, E_1, E_2, $F_{2\alpha}$, (15s)-15 methyl E_2, and 16, 16-dimethyl E_2 (courtesy of

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Dr. John E. Pike, Upjohn Company, Kalamazoo, Mich.). Stock solutions for the nonmethylated prostaglandins were diluted to a concentration of 10 mg/ml in absolute ethanol. Similar dilution of the methylated derivatives was accomplished using olive oil. Further dilution of all stock solutions to the desired dose was also done with olive oil. Dosages were calculated to exceed the minimum effective dose (MED) reported by Dukes *et al.* (7) by 20% with the exception of prostaglandins A_1 and $F_{2\alpha}$ which were administered in 375 μg/100 g body weight doses. All other prostaglandins were injected in 125 μg/100 g body weight aliquots into male Long–Evans rats (175–200 g). Four sets of experiments were performed:

1. The effect of the prostaglandins on Ep production in renal-intact rats. Normal rats were injected ip with each of the prostaglandins followed 24 hr later by hypoxia (0.4 atm/6 hr) in a decompression chamber. At the termination of hypoxia, rats from each group were exsanguinated from the abdominal aorta, their sera pooled, and assayed for Ep in the exhypoxic polycythemic mouse (13).

2. The effect of prostaglandins on hepatic Ep elaboration. Two groups of animals were studied. (A) Normal rats were injected ip with each of the prostaglandins 24 hr prior to bilateral nephrectomy (nephrx) and exposure to hypoxia (0.4 atm/6 hr). After hypoxia, sera from each group was pooled and assayed for Ep (13). (B) Prostaglandins were injected ip immediately after hepx (14). Nephrx and hypoxia were performed 24 hr later. Sera from each group was pooled and assayed for Ep.

3. The effect of prostaglandins on Ep production by the regenerating liver. Rats were 80–90% hepx using a method described previously (14). The livers were allowed to regenerate for 72 hr prior to nephrx, hypoxia, and Ep assay (13). Prostaglandins were injected ip 24 hr before nephrx, i.e., at 48 hr after hepx. Regenerating liver has been reported to produce significant quantities of Ep in response to hypoxia (14, 15). The peak hepatic Ep response to hypoxia occurred at 72 hr post-hepx (14). The ability of the prostaglandins to augment this response was studied in this experiment.

4. Controls. Three sets of control groups were employed: (a) room pressure (1 atm/6 hr) was substituted for hypoxia for each of the protocols listed in the first three groups; (b) animals were injected with the vehicle used for each prostaglandin (ethanol/olive oil or olive oil) at the same time intervals as previously described; (c) rats were injected with saline instead of test material or vehicle as described for the first three groups.

Each experiment was performed three or four times using five to eight rats per trial. Ep levels were determined using CF-1 virgin ♀ mice which were subjected to discontinuous hypoxia at 0.4 atm of air for 19 hr/day for 2 weeks rendering them polycythemic. Exhypoxic polycythemic animals are extremely sensitive to Ep in the donor sample since they produce little or no endogenous Ep. These mice were injected with 1 ml of test plasma at 3 days after the termination of hypoxia. On Day 5 posthypoxia, the mice were given an iv injection of radioiron and were exsanguinated 2 days later via cardiac puncture. Percentage red blood cell radioiron incorporations were determined for each of the test samples and compared to those obtained with standards derived from the international reference preparation for Ep. Each sample point was assayed in four or five mice (13).

Results. In all of the experiments, the synthetic methylated prostaglandins (PG 16,16-dimethyl E_2 and (15s)-15 methyl E_2) produced the most potent Ep effect (Figs. 1–5). The Ep response to hypoxia in the intact rat was enhanced by treatment with PGs 16,16-dimethyl E_2, (15s)-15 methyl E_2, E_2, and A_2, was unaffected by the administration of PG A_1 or E_1 in the dose used, and was inhibited approximately 40% by $PGF_{2\alpha}$ (Fig. 1; $P < 0.025$). These cyclic compounds appear to exert an effect on extrarenal Ep production as well (Fig. 2). When normal rats were administered PG (15s)-15 mE_2, 16,16 mE_2, E_2, A_2, or E_1 24 hr prior to nephrx and hypoxic exposure, they produced significantly greater quantities of Ep when compared to the vehicle or saline-injected controls (range of $P < 0.01$ to $P <$

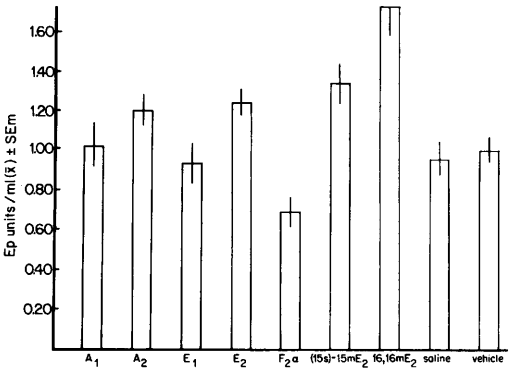

FIG. 1. The effects of various prostaglandins on the Ep response to hypoxia in the intact rat. The levels of significance of individual groups compared to saline- or vehicle-injected controls: 16,16mE_2, (15s)-15mE_2, $P < 0.025$; A_2 and E_2, $P < 0.05$; A_1 and E_1, not significant; $F_{2\alpha}$, $P < 0.025$. Vertical lines through the bars represent ± standard error of the mean. The levels of significance (P values) in this and subsequent groups were determined using Student's t test.

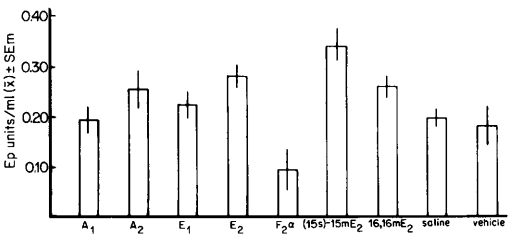

FIG. 3. The effects of various prostaglandins on Ep elaboration after hypoxia in the hepx renoprival rat. The levels of significance of individual groups compared to saline or vehicle injected controls: (15s)-15mE_2, 16,16mE_2, and E_2, $P < 0.05$; A_2, E_1, A_1, and $F_{2\alpha}$, not significant.

0.05; Fig. 2). PG A_1 and $F_{2\alpha}$ displayed no effect on extrarenal Ep production at the dose level used (Fig. 2). The PGs did not exert a significant effect on Ep elaboration in the hepx renoprival hypoxic animal (Fig. 3) although some augmentation of Ep levels were noted with PG (15s)-15 methyl E_2($P < 0.05$). The PGs were also tested in the regenerating liver model. Hepatic Ep production in response to hypoxia is highest at 72 hr after hepx (14, 15). PGs were injected at 48 hr post-hepx in an attempt to further stimulate hepatic Ep production. Enhancement of Ep elaboration in response to hypoxia by liver regenerating 72 hr after hepx was observed 24 hr following the administration of PG (15s)-15mE_2, 16,16mE_2, and A_2($P < 0.025$, $P < 0.01$, and $P < 0.05$ respectively; Fig. 4) when compared to saline- or vehicle-injected controls. A significant inhibition of hepatic Ep was noted when hepx animals were injected with PG $F_{2\alpha}$ at 24 hr prior to nephrx and hypoxia ($P < 0.01$) (Fig. 4). In prostaglandin-treated renal intact rats with livers regenerating 72

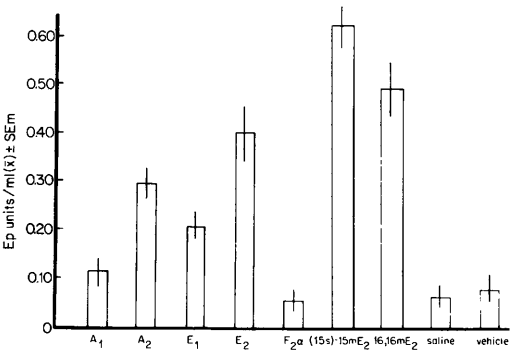

FIG. 2. The effects of various prostaglandins on Ep production in the anephric hypoxic animal. The levels of significance of individual groups compared to saline or vehicle injected controls: (15s)-15mE_2, $P < 0.01$; 16,16mE_2 and E_2, $P < 0.025$; A_2 and E_1, $P < 0.05$; A_1 and $F_{2\alpha}$, not significant.

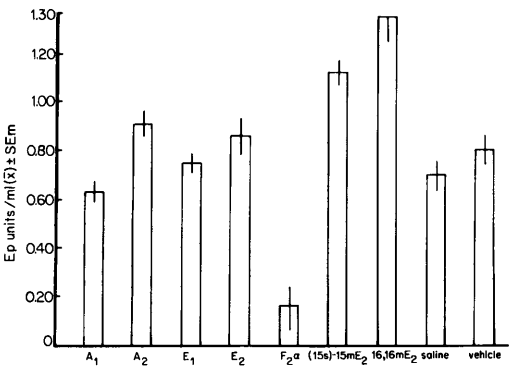

FIG. 4. The effects of various prostaglandins on Ep production in anephric hypoxic rats with livers regenerating for 72 hr after hepx. The levels of significance of individual groups compared to saline- or vehicle-injected controls: 16,16mE_2 and $F_{2\alpha}$, $P < 0.01$; (15s)-15mE_2, $P < 0.025$; A_2, $P < 0.05$; A_1,E_1, and E_2, not significant.

hr after hepx, the Ep response to hypoxia was similar in magnitude to that seen in normal prostaglandin-injected animals (Fig. 5 vs Fig. 1). Some diminution in the Ep levels of these hepx, renal-intact hypoxic rats was noted after treatment with PG $F_{2\alpha}$ (Fig. 5; $P < 0.05$). The greatest Ep response to hypoxia in animals with intact kidneys was manifested after injection of PG 16,16mE_2 (Figs. 1 and 5). In contrast, regardless of the condition of the liver, Ep levels after hypoxia were highest in renoprival animals treated with PG (15s)-15mE_2 (Figs. 2–4; $P < 0.05$ when compared to PG 16,16mE_2, and $P < 0.025$ to $P < 0.01$ when compared to the other prostaglandins). All Ep stimulatory effects required exposure to hypoxia; ambient (room) pressure controls manifested no significant Ep response following prostaglandin treatment (Table I).

The circulating half-lives of some of the PGs used in this study (PG E_1, E_2, A_2, and F_2) are less than one minute in duration, although their primary metabolites (15-keto and 15-keto, 13,14-dihydro compounds) last considerably longer and although lessened, exhibit some biological activity (26). In contrast, the synthetic methylated derivatives are resistant to prostaglandin dehydrogenase, and therefore remain in the circulation substantially longer (22). The effects of these compounds on erythropoiesis and Ep production, however, are not manifested immediately following administration (2, 3, 19) and thus a lag time is required prior to measurement of these parameters.

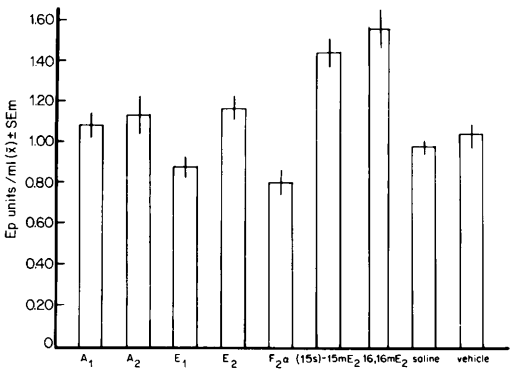

FIG. 5. The effects of various prostaglandins on the Ep response to hypoxia in renal-intact hypoxic rats with livers regenerating 72 hr after hepx. The levels of significance of individual groups compared to saline- or vehicle-injected controls: 16,16mE_2, $P < 0.025$; (15s)-15mE_2 and $F_{2\alpha}$, $P < 0.05$; E_1, E_2, A_1, and A_2, not significant.

Discussion. Prostaglandin stimulation of erythropoiesis has been attributed to a twofold effect: stimulation of Ep synthesis and increasing the responsiveness of stem cells to Ep (4, 6, 7). The renovascular effects of the E and A series prostaglandins have been cited as a possible cause of this elevated Ep response (3, 9), although this is a species-specific action (9). An alternative hypothesis was that prostaglandins, prostacyclins, or their derivatives stimulate cAMP, which, in turn, causes augmented Ep production (4, 7, 8). Prostaglandin effects on hematopoietic cells appear to be more clear-cut: members of the E and A series prostaglandins, which stimulate erythropoiesis, were found to inhibit *in vitro* granulopoiesis (16, 17). Likewise, prostaglandin $F_{2\alpha}$, which inhibits erythropoiesis, actually stimulated the growth of cultured granulocyte–macrophage progenitor cells (5). The role of these cyclic compounds in modulating hematopoiesis is as yet uncertain; further experimentation is required to elucidate their precise mechanism of action.

Although the influence of prostaglandins on renal physiology and Ep production has been well documented (1, 3, 9, 18), little information exists concerning the effects of these compounds on extrarenal Ep (12). Increased radioiron uptake into red blood cells of exhypoxic polycythemic mice was reported after treatment with prostaglandins A_2, (15s)-15mE_2, or 16, 16mE_2, regardless of the presence of the kidney (12), but it was unclear to what extent Ep synthesis was enhanced. In the present study, the synthetic methylated prostaglandins (16, 16mE_2, and (15s)-15mE_2) induced the highest Ep levels in response to hypoxia in normal, anephric, and hepx rats (Figs. 1–5). Prostaglandins E_2 and A_2 stimulated Ep production in anephric and renal-intact hypoxic rats if the livers of these animals were either normal or allowed to regenerate for 72 hr after hepx (Figs. 1, 2, 4, 5). With the exception of prostaglandin (15s)-15mE_2, none of the other agents evoked an Ep re-

TABLE I. MEAN Ep units/ml ± 1 STANDARD ERROR OF THE MEAN IN THE SERUM OF RATS INJECTED[a] WITH VARIOUS PROSTAGLANDINS AND SUBJECTED TO ROOM PRESSURE (1 atm/6 hr)

	A_1	A_2	E_1	E_2	$F_{2\alpha}$	$15mE_2$	$16,16mE_2$
Normal	[b]	0.06 ± 0.01	[b]	0.05 ± 0.01	[b]	0.05 ± 0.02	0.05 ± 0.02
Normal-nephrx 24 hr	[b]	[b]	[b]	0.05 ± 0.02	[b]	0.12 ± 0.03	0.06 ± 0.02
Hepx → nephrx 72 hr	[b]	[b]	[b]	[b]	[b]	0.06 ± 0.02	[b]
Hepx → nephrx	0.05 ± 0.01	0.06 ± 0.02	0.05 ± 0.02	0.07 ± 0.01	[b]	0.06 ± 0.01	0.06 ± 0.01

[a] In nephrx animals, prostaglandins were injected 24 hr prior to surgery. In the normal-intact animal, a total time of 30 hr elapsed between prostaglandin injection and exsanguination.
[b] Undetectable in the exhypoxic polycythemic mouse assay.

sponse after hypoxia in the acute hepx (24 hr), anephric rat (Fig. 3). It appears that these prostaglandins affect not only renal Ep production but the extrarenal elaboration of this principle as well. Removal of approximately 80–90% of the hepatic mass 24 hr prior to nephrx and hypoxia abolished the extrarenal Ep-stimulatory action of all prostaglandins except (15s)-$15mE_2$ (Fig. 3). However, after the administration of this prostaglandin, the acutely hepx (24 hr) anephric rat responded to hypoxia with approximately 50% less Ep than the liver-intact renoprival animal (Fig. 2 vs Fig. 3) and about 75% less Ep than the hepx anephric rat whose liver was allowed to regenerate for 72 hr prior to exsanguination (Fig. 3 vs Fig. 4). The elevation in extrarenal Ep levels after treatment of the acutely hepx (24 hr) animal with prostaglandin (15s)-$15mE_2$ may be explained by: (i) stimulation of a site of extrarenal Ep production other than the liver or (ii) acting on the liver remnant which is approximately 20% regenerated at 24 hr following surgery (14). Since the administration of prostaglandin (15s)-$15mE_2$ evoked an Ep response in the 72-hr post-hepx rat which was proportional to the observed increase in regenerated liver mass, the latter possibility is favored. Like these methylated compounds, the naturally occurring prostaglandins (E_2, A_2) also were capable of augmenting Ep elaboration after hypoxia in the liver-intact or 72-hr post-hepx regenerating liver, renoprival rat (Figs. 2, 4). Another naturally occurring prostaglandin, E_1, displayed a baseline stimulation of extrarenal Ep in response to hypoxia (Fig. 2) although no significant action of this compound on Ep production in renal-intact hypoxic animals was observed (Fig. 1). An alteration of normal prostaglandin metabolism in hepx, nephrx, or combined hepx and nephrx rats may have some influence on the results. Catabolism of the E and A series prostaglandins occurs in part through the action of prostaglandin dehydrogenase, which has been localized in the lungs and kidney (1, 19). Degradation of these compounds occurs in the liver, via B oxidation (20). The pronounced Ep stimulatory effects of the synthetic methylated prostaglandins (21) have been attributed to their resistance to inactivation by prostaglandin dehydrogenase (22). They are apparently somewhat resistant to B oxidation as well, since their effects on Ep in the normal, renal-intact hypoxic animal were similar to that observed in hepx renal-intact hypoxic rats (Fig. 1 vs Fig. 5).

It is probable that these prostaglandins also stimulate cells which produce Ep. Macrophages have been implicated in the production of E series prostaglandins (17, 23) and colony stimulating factor (CSF) (24), as well as Ep (14). Different macrophage subpopulations have been reported to produce prostaglandin E and CSF (25), which exert opposite effects on erythroid and granuloid hematopoietic stem cells (8, 17). Macrophage-derived prostaglandin E has also been cited as a possible modulator of hematopoiesis, through its ability to enhance *in vitro* erythroid growth (8) and inhibit the maturation of granuloid elements

in culture (17). Several macrophage subpopulations may exist and modulation of hematopoiesis may, at least in part, occur through the production of various prostaglandins by macrophages which potentiate hematopoietic stem cells to respond to Ep or CSF or stimulate other macrophage populations to produce Ep or CSF. The present report indicates that extrarenal Ep production is stimulated by agents known to act on the renal elaboration of this factor. The liver appears to be the primary site of extrarenal Ep stimulation by prostaglandins, possibly because of its relatively large macrophage population.

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