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The Effect of Aspirin on Erythropoietin Formation in the Rat (40577)[1]

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It is well documented that the kidney represents the main site of erythropoietin (Epo) production or activation, and that hypoxia stimulates Epo production (1). However, the precise mechanism of hypoxia-induced Epo production is still not elucidated. Several reports have recently indicated the possible role of renal prostaglandins (PG) in this phenomenon. Thus, it has been reported that PGs of the A and E series stimulate Epo production of mice (2–4) and in isolated perfused dog kidney (2, 4). Moreover, an increased renal efflux of PGs and Epo, induced in dogs by either acute hypoxia or renal artery constriction, can be blocked by the PGs synthesis inhibitor—indomethacin (5–7). It has also been reported that packed cell volume (PCV) in chronically hypoxic rats treated with aspirin (an inhibitor of PGs synthesis) is significantly lower than in chronically hypoxic, otherwise untreated, rats (8).

The purpose of the present study was to further investigate the role of PGs in Epo formation, by examining the effect of inhibition of PGs synthesis on Epo production in rats exposed to a hypoxic stimulus.

Materials and methods. The experiments were performed using adult male rats, weighing 200 to 300 g. Two different strains of rats were used: a substrain of Wistar rats bred at our institution for several generations and a strain of rats bearing congenital hydronephrosis (MRC/H rats), originally obtained from the Memorial Research Center, Knoxville, Tennessee, and bred at our Institute. The main feature of hydronephrosis in MRC/H rats is extensive destruction of renomedullary tissue, the renal cortex being less involved. A detailed description of MRC/H rats has been given elsewhere (9, 10). Diagnosis of kidney disease was made at autopsy. Since the kidney medulla is the major source of renal PGs, it was of interest to study Epo formation in animals in which the renal medulla, and presumably PGs synthesis, were affected.

Rats of both strains were divided into two groups, one control and the other aspirin treated. All animals were allowed water and food *ad libitum*. The control groups were given tap water, while experimental groups received water in which acetylsalicylic acid (4 g/liter) was dissolved. The average daily water intake, in both Wistar and MRC/H rats, was 20 ml per rat, so that calculated mean aspirin intake was 80 mg per rat. After 4 days of their respective treatments, the rats were exposed to a hypoxic stimulus. To this end, animals (kept in individual cages) were housed in a large "hypoxic cage," an airtight plastic cage with walls partially replaced with dimethylsilicone rubber membranes (General Electric, Schenectady, N.Y.) with a defined permeability for O_2 and CO_2 (11). The number of rats in the hypoxic cage was adjusted so that the O_2 content in the cage reached 7%. The animals were kept in the hypoxic cage for 24 hr, the O_2 content being around 7% during the last 10 hr. Immediately after exposure to hypoxia, the animals were anesthetized with ether, and blood samples for the determination of serum Epo activity and PCV were obtained through a needle placed into the abdominal aorta. Only the animals with PCV over 40% were used. For the determination of serum Epo activity, serum was separated and samples from all rats in the same group were pooled and stored at −20° until assayed.

Animals from all groups were placed in the hypoxic cage simultaneously, and the experiment was repeated three times. Results for each group, obtained on three different occasions, were pooled and expressed as mean ± SE. Statistical analyses (t test) were per-

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formed according to Steel and Torrie (12).

The Epo level in the sera was indirectly determined by measuring the 48-hr ^{59}Fe incorporation into red blood cells (RBC) in mice with posthypoxic polycythemia, as previously described (13). On the sixth and seventh posthypoxic day mice were injected i.p. (0.5 ml per mouse) with either saline (negative control), two doses of Epo standard, or the rat serum. Each serum sample was tested in at least five mice.

Results. Body weight was found to be similar in experimental and control groups in both strains of rats (240 ± 12 g in control Wistar, 236 ± 14 g in aspirin-treated Wistar rats, 248 ± 11 g in control MRC/H rats, and 251 ± 12 g in aspirin-treated MRC/H rats). Similarly, no difference in PCV was found between the groups (41.8 ± 0.9% vs 41.8 ± 0.6% in control and aspirin-treated Wistar rats, and 43.0 ± 0.8 vs 41.3 ± 0.6% in control and aspirin-treated MRC/H rats).

Figure 1 shows the serum Epo activity (expressed as 48-hr percentage ^{59}Fe RBC incorporation) in control and aspirin-treated rats. Significantly lower ($P < 0.01$) serum Epo activity was found in aspirin-treated Wistar rats when compared to the respective control animals. However, no difference in serum Epo activity was found between aspirin-treated and control MRC/H rats. The 48-hr ^{59}Fe RBC incorporation in three different experiments is shown in Table I.

Discussion. The presented results show that aspirin effectively diminishes Epo production in Wistar rats exposed to a hypoxic stimulus. Assuming that the effects of aspirin are mediated through the inhibition of PGs synthesis (14) this finding inferentially supports the suggestion (5–7) that PGs mediate Epo production by the kidney.

TABLE I. THE PERCENTAGE 48-H ^{59}Fe RBC INCORPORATION IN THREE DIFFERENT EXPERIMENTS

Groups	Experiments I	II	III
Wistar rats			
Control	9.3 ± 0.9	16.9 ± 1.8	14.1 ± 1.1
Aspirin	7.0 ± 1.4	6.2 ± 1.0	4.6 ± 0.3
MRC/H rats			
Control	5.7 ± 0.6	11.9 ± 0.9	14.8 ± 1.1
Aspirin	7.7 ± 0.6	10.1 ± 1.3	15.2 ± 1.1

In respect to the aforementioned data, somewhat puzzling and contradictory are the results showing no difference in serum Epo activity between control and aspirin-treated rats with hereditary hydronephrosis. It may be speculated that not only species, but also a strain difference exists in factors controlling Epo formation (15). Furthermore, since renal PGs are mostly produced by the kidney medulla (16) and since the renal medulla is grossly destroyed in hydronephrotic rats, it may be assumed that PGs synthesis in hydronephrotic kidneys is likewise greatly diminished. In this context, the failure of aspirin to diminish Epo formation in hypoxic MRC/H rats can be easily explained.

It should be noted that the presented results show no difference in Epo formation between

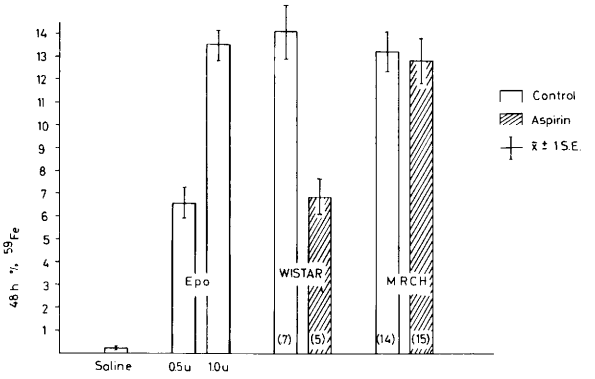

FIG. 1. The 48-hr^{59}Fe incorporation into red blood cells in exhypoxic polycythemic mice injected with either saline (negative control), two doses of erythropoietin (Epo) standard, or rat serum. The number of animals in each group is given at the bottom of the respective column.

control Wistar and control MRC/H rats. Moreover, Lozzio *et al.* (17) have already reported that severe hydronephrosis does not affect the ability of rats to produce Epo or to maintain normal erythropoiesis. Assuming that, due to medullary damage, PGs synthesis is greatly diminished in hydronephrotic kidneys, both findings would, by inference, lead to the conclusion that PGs are neither the sole nor an essential mediator of hypoxia-induced Epo formation, and that other factors may compensate for PGs deficiency. The suggestion that PGs are not the sole mediator of Epo production is also supported by the previously reported results (8) that in chronically hypoxic Wistar rats treated with aspirin the PCV, although lower than in the appropriate controls, is significantly greater when compared to that of untreated rats (PCV, 42, 82, and 64% in normoxic, chronically hypoxic, and chronically hypoxic rats treated with aspirin, respectively). Furthermore, it is not known whether a number of factors, i.e., salt intake, which influence renal PGs efflux (18) change Epo formation by the kidney.

Summary. The effect of aspirin on Epo formation was studied in normal Wistar rats and rats with hereditary hydronephrosis in which, due to renomedullary damage, PGs synthesis by the kidney is supposedly diminished. The obtained results show that, in Wistar rats exposed to a hypoxic stimulus, aspirin effectively diminishes Epo formation, presumably by inhibiting PGs synthesis. Under the same conditions aspirin failed to abolish Epo production in rats with hereditary hydronephrosis. No difference in Epo formation was found between control Wistar and control hydronephrotic rats when exposed to a hypoxic stimulus. The presented results indicate that renal PGs are a contributory factor in the mechanism of hypoxia-induced Epo production, rather than the controlling one.

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