Erythropoietic Effects of 5-Hydroxytryptamine[1] (35840)

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Several types of pharmcological agents have been demonstrated to stimulate erythropoietin (ESF) production. Most of these compounds have been found to exert their effects through a renal hypoxic mechanism by decreasing renal blood flow (1–4), increasing oxygen demand (5), or by interfering with the utilization of oxygen by producing renal histotoxic hypoxia (5, 6).

The vasoactive agents, which have been found to stimulate ESF production through an alteration in renal hemodynamics, are pressor substances such as catecholamines (7, 8) and angiotensin (1, 2, 4). Mechanical constriction with the use of a modified Goldblatt clamp (1, 3) has also been demonstrated to elevate plasma ESF titers. Serotonin (5-hydroxytryptamine) is known to alter renal function in several laboratory animals (9). Renal ischemia and necrosis have been reported (9) in rats and rabbits following subcutaneous injections or large doses of 5-hydroxytryptamine (5-HT).

5-HT was reported recently to produce an elevation in hemoglobin and reticulocyte levels in the peripheral blood of rabbits (10) and to increase the incorporation of radioactive iron (^{59}Fe) in red cells of polycythemic mice (11, 12). The mechanism of the erythropoietic action of 5-HT is not known. The present studies attempt to relate the erythropoietic effects of 5-HT to enhanced renal erythropoietin production.

Materials and Methods. The polycythemic mouse assay (13) was used to determine plasma erythropoietic activity. Endogenous production of erythropoietin is suppressed in exhypoxic polycythemic mice rendering them more sensitive to small amounts of exogenous erythropoietin. HA/ICR strain female mice (18–25 g) were made polycythemic by exposure to 0.42 atm for 2 weeks. The mice were injected subcutaneously with 1/2 the total dose of either saline, human urinary erythropoietin,[2] or the test substance on the fourth and fifth days following removal from the hypobaric chamber. Each mouse received 0.5 μCi of ^{59}Fe citrate iv on the sixth posthypoxic day. Two days later (posthypoxic day 8) each animal was exsanguinated via cardiac puncture and the percentage ^{59}Fe incorporation in red blood cells determined.

Female mongrel dogs (14–20 kg) were anesthetized intravenously with sodium pentobarbital (30 mg/kg) and the mean carotid artery blood pressure was measured with a Statham pressure transducer and a Grass Polygraph. Heparinized saline (50 U/ml) was used as the anticoagulant to rinse the syringe used to withdraw the blood samples. A polyethylene catheter was passed up the right femoral artery into the abdominal aorta to a position a few centimeters above the branches of the renal arteries to afford the kidneys a high concentration of the substance being infused. A Harvard pump was employed to infuse 5-hydroxytryptamine (21.75 μg/kg/min) as the creatinine sulfate salt.[3] Creatinine sulfate alone in saline was infused in control dogs. The dosage of creatinine sulfate was equivalent to the amount of creatinine salt contained in the 5-HT infusions.

A second polyethylene catheter was inserted into the left femoral vein to remove blood samples at various time intervals. Microhe-

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[2] The erythropoitein used was a freeze-dried preparation from the urine of a patient with a pure red cell aplasia.

[3] 5-Hydroxytryptamine creatinine sulfate was obtained from the Nutritional Biochemical Company, Cleveland, Ohio.

matocrit determinations were performed on the blood samples removed at 0, 2, 3, 5, and 6 hr of infusion. The blood samples were centrifuged at 2000 rpm for 15 min, plasma removed, and frozen at —84° until assayed for erythropoietic activity in polycythemic mice. Blood flow was measured with a Sine Wave Biotronics electromagnetic flowmeter. The electromagnetic probe (3.0-mm diameter lumen) was placed on the left renal artery through a retroperitoneal incision.

To determine whether the effect of 5-HT on ^{59}Fe incorporation is erythropoietin dependent, polycythemic mice were treated subcutaneously with antiserum to erythropoietin (Anti-ESF) and 5-HT simultaneously. The erythropoietin antiserum was prepared by injecting New Zealand albino rabbits with the freeze-dried extract of crude human urinary

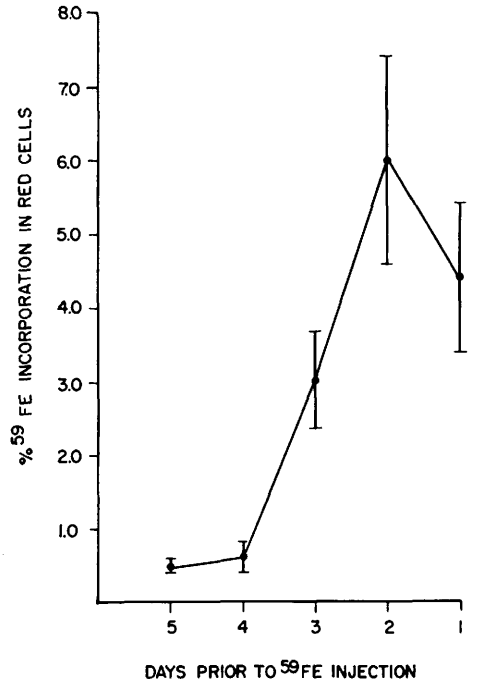

FIG. 1. Mean radioactive iron incorporation in red cells of polycythemic mice injected with 5-hydroxytryptamine at several time intervals prior to ^{59}Fe injection. A total dose of 350 mg/kg of 5-hydroxytryptamine, sc, was injected into 8–10 polycythemic mice. Each injection of 5-HT was divided into two equal doses separated by an 8-hr interval. Standard error of the mean is shown for each point.

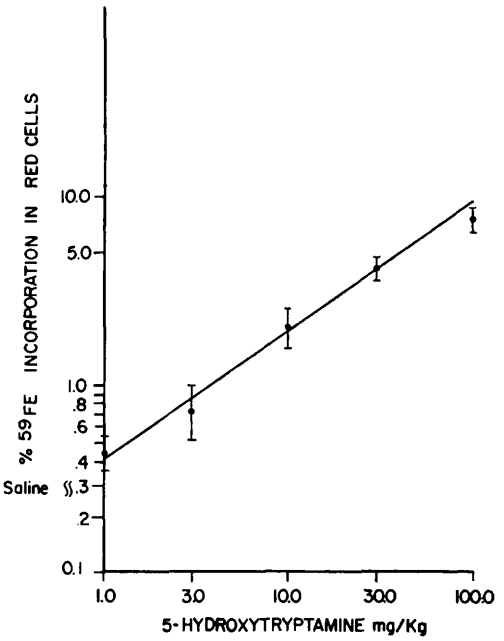

FIG. 2. Dose–response regression line for 5-hydroxytryptamine on radioiron incorporation in red cells of polycythemic mice. The 5-HT was injected on the fourth posthypoxic day under same schedule as indicated in Fig. 1. Each point with its standard error represents the mean ^{59}Fe incorporation of 10–16 mice.

erythropoietin according to a modification of the technique of Kabat and Mayer (14). The immunization schedule consisted of sc injections of erythropoietin (total 157.6 units) in complete Freunds adjuvant 3 times/week for 4 weeks. On days 5 and 6 after the final injection, 15 ml of blood were removed via cardiac puncture, serum was collected and tested against human urinary ESF for neutralizing activity in polycythemic mice. Each injection of 5-HT was divided into two equal doses separated by an 8-hr interval. The antiserum (0.1 ml) was injected (sc) into mice at 0, 8, 12, 22, 32, and 36 hr following the injections of 5-HT. Thus, a total of 0.6 ml of anti-ESF was administered. Anti-ESF was incubated with ESF for 30 min at 37° prior to being injected into the assay mice.

Results. Figure 1 illustrates that the maximum effect of 5-HT on percentage ^{59}Fe incorporation into red cells occurred when 5-HT was injected 48 hr prior to the injection of radioiron. This time sequence is prob-

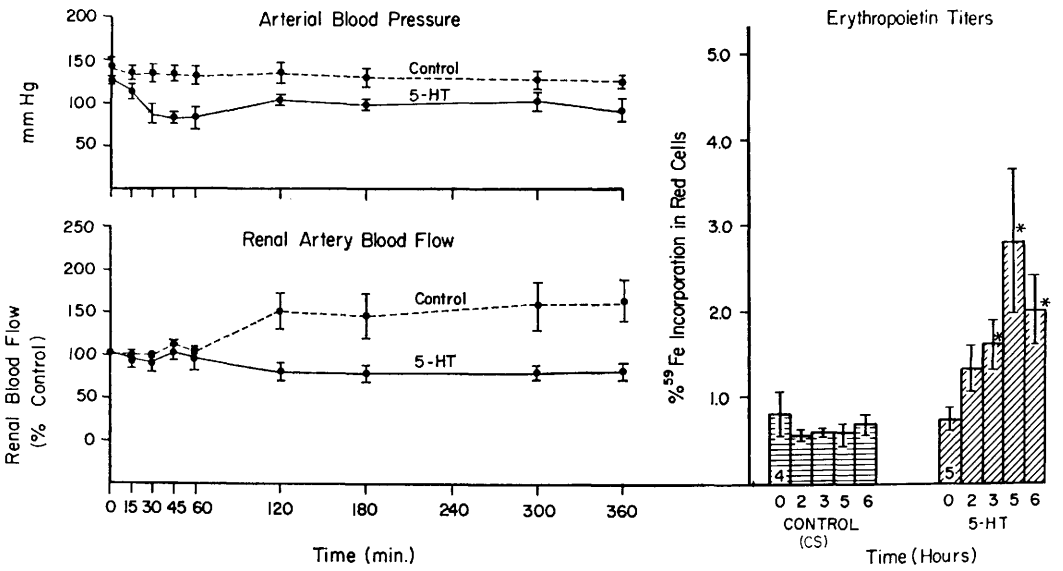

FIG. 3. Effects of 5-hydroxytryptamine (5-HT) in 5 dogs and creatinine sulfate (CS) in 4 dogs on arterial blood pressure, renal blood flow, and plasma erythropoietic activity. Each mouse received 0.5 ml of dog plasma on the fourth and fifth posthypoxic days (total 1.0 ml of plasma). Asterisk indicates significantly different ($p<.05$) when compared to the 0-hr value.

ably correlated with a rapid release of erythropoietin 2 days before its peak effect on ^{59}Fe incorporation. Figure 2 demonstrates a linear log-dose log-response relationship with dosages of 5-HT between 1–100 mg/kg when the dose–response regression line was fitted by the method of least squares (15). Figure 3 shows the changes in arterial blood pressure, renal blood flow, and plasma erythropoietic activity during infusion of 5-hydroxytryptamine creatinine sulfate (5-HT) or creatinine sulfate (CS) alone in dogs. There was no significant change in blood pressure during the control infusions with creatinine sulfate. However, an early fall in blood pressure from an initial value of 130 mm Hg to a value of 83 mm Hg during 5-HT infusions was seen. The blood pressure returned to a value of approximately 110 mm Hg after 2 hr. The renal blood flow (RBF) during the creatinine sulfate infusion was relatively stable for the first 60 min but gradually increased to a plateau of 150% of the initial value and remained elevated throughout the remainder of the experiment. An increase in renal flow has been reported previously when saline was infused in a similar manner (16). The 5-hydroxytryptamine infusion resulted in a gradual fall in RBF which was reduced to approximately 75% of the initial value after 360 min.

As noted in the bar graphs in Fig. 3, plasma erythropoietic activity was not significantly elevated above the 0 time value after 2, 3, 5, and 6 hr infusion with creatinine sulfate. However, the plasma erythropoietic activity was significantly increased above the initial 0 time value after 3, 5, and 6 hr infusion with 5-HT, when compared with the use of Dunnett's test for multiple comparisons with a single control (17).

Table I illustrates the effect of erythropoietin antiserum (Anti-ESF) in polycythemic mice treated with erythropoietin or 5-hydroxytryptamine. 0.1 ml of the antiserum completely blocked the effects of 0.2 u ESF in polycythemic mice. When 5-HT was used as the test substance, in a total dose of 350 mg/kg, a significant ($p<.05$) reduction in the erythropoietic activity was seen when 5-HT was injected concurrently with Anti-ESF. However, significant erythropoietic activity was still present after the combination of Anti-ESF and 5-HT. Thus, it appears that the response to 5-HT is not completely erythropoietin dependent.

TABLE I. Inhibitory Effects of Anti-ESF on Erythropoietin and 5-HT in Polycythemic Mice.

Treatment	No. of mice	Mean (%) ^{59}Fe incorporation into RBC (± SE)[a]
Saline	15	0.42 ± 0.13
ESF 0.2 units	14	7.19[b] ± 0.88
5-HT (350 mg/kg) + saline	15	8.24[b] ± 2.43
5-HT (350 mg/kg) + anti-ESF	14	1.75[b, c] ± 0.58
ESF (0.2 units) + 0.1 ml of anti-ESF	5	0.29 ± 0.02

[a] Standard error of the mean.

[b] Significantly different from saline control ($p < .05$).

[c] Significantly different from ESF (0.2 units) and 5-HT ($p < .05$).

Discussion. It has been reported previously that renal blood flow in the dog must be reduced to a range of 40–60% of the initial value before a sharp rise in plasma levels of erythropoietin occurs (3). In addition, a 30% reduction in renal flow was not sufficient to produce the magnitude of change in plasma levels of erythropoietin which was seen with 5-HT infusions in the present studies. Therefore, the reduction of 25% in RBF seen with 5-HT infusions in our experiments would not appear to be sufficient alone to cause the observed elevation in plasma erythropoietic activity. On the other hand, if the percentage decrease in renal blood flow is compared with the mean elevation in renal flow observed with the control creatinine sulfate infusion, a 50% reduction in RBF was noted after 6 hr. This may be sufficient to cause erythropoietin elaboration due to renal ischemia. However, it is important to note that the net change in RBF following 5-HT infusion was a decrease of approximately 25%. It is also quite possible that 5-HT could have produced a selective effect on renal vessels within the kidney to cause a more marked intrarenal vasoconstriction near the site of erythropoietin elaboration than that reflected in the measurement of blood flow directly through the renal artery.

According to Thaysen *et al.* (18) when renal blood flow falls below a value of 1 ml/g/min, an increase in the A-V_{O_2} difference is seen. Normally, at flows above 1 ml/g/min, the A-V_{O_2} difference in the kidney remains relatively stable. Fisher and Samuels (3) reported that the most marked increase in ESF elaboration was seen when RBF was reduced by 65–78% which was below 1 ml/g/min. They suggested that this percentage reduction in RBF was sufficient to produce the degree of renal hypoxia necessary to stimulate erythropoietin production. Thus, the decrease in RBF seen in our studies would not appear to be sufficient to stimulate ESF production and a different mechanism of action for 5-HT on erythropoietin production is suggested.

There was no correlation found between changes in renal blood flow and erythropoietic activity in plasma during 5-HT infusion when individual experiments were compared. Likewise, antiserum to erythropoietin did not block completely the erythropoietic effect of 5-HT in polycythemic mice. Thus, it would appear that even though the primary effect of 5-HT is through enhanced erythropoietin production and/or release of erythropoietin, there is probably a second effect of 5-HT which does not depend on elaboration of erythropoietin. It is quite possible that the residual erythropoietic effect on 5-HT in polycythemic mice is due, at least in part, to changes in iron kinetics or to a direct effect on erythroid cells in the bone marrow.

Summary. Serotonin (5-hydroxytryptamine) was found to produce a significant increase in radioiron incorporation in red cells of polycythemic mice. A linear dose–response relationship is reported. Anti-ESF produced a significant inhibition of the erythropoietic effects of 5-HT in polycythemic mice. However, residual erythropoietic activity was still seen even in the presence of antiserum to erythropoietin suggesting a secondary erythropoietic effect which is not erythropoietin dependent. Intrarenal infusions of 5-HT into dogs was found to produce a significant ele-

vation in plasma erythropoietic activity.

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