Relationship Between Renal Blood Flow and Erythropoietin Production In Dogs.* (32125)

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Erythropoietin production has been found to be increased following renal artery constriction in rabbits and rats(1,2,3). However, the per cent reduction in renal blood flow necessary to stimulate erythropoietin (ESF) elaboration is not known. Renal blood flow has not been reported in any of the studies on erythropoietin formation following renal artery constriction. In order to determine the approximate level of reduction in renal blood flow necessary to stimulate ESF production, plasma levels of ESF were measured in dogs following graded decreases in renal blood flow by constricting the renal artery with a modified Goldblatt clamp. Renal blood flow was reduced initially to 71, 50 and 35.4% of preconstriction values and plasma erythropoietin titers measured at several time intervals over a 96 hour period.

Materials and methods. Male and female mongrel dogs were used throughout these studies. Renal artery blood flow (RBF) was measured directly with an Electromagnetic Flowmeter. The probe (5-7 mm) was placed on the left renal artery following exposure through a midline abdominal incision while the dog was anesthetized with pentobarbital. A modified Goldblatt clamp was placed on the left renal artery and the renal arteries constricted initially to levels of blood flow approximating 71, 50 and 35.4% of preconstriction values. RBF was monitored during constriction with the probe placed distal to the clamp. The blood flow was measured for a period of 15-20 minutes following placement of the clamp in order to insure that the desired degree of reduction in blood flow had been maintained. Approximately 40 ml of blood was removed from the unanesthetized dog *via* direct femoral artery puncture initially and 6, 12, 24, 48, and 96 hours following placement of the clamp. The probe was placed on the constricted artery again at the end of 96 hours and renal blood flow measured to determine the change in RBF since the initial measurement. The dogs were sacrificed at the end of 96 hours and both kidneys removed for histological studies. The procedure for the sham operated dogs was exactly the same with the exception that the clamp was not placed on the artery following the initial measurement of RBF.

The dog plasma samples were assayed in polycythemic mice *via* a modification of the method of DeGowin *et al*(4). Male Swiss-Webster strain mice were injected with 1 mg of iron dextran prior to being placed in a low pressure tank at 0.45 atmosphere for 3 weeks. On the day the mice were removed from the tank each mouse was injected with 1 ml donor mouse blood at hematocrit 70. On the fifth day out of the tank each mouse was injected with 1 ml of dog plasma 3 times at 5 hour intervals. Control mice were injected with saline and a standard group of mice were injected with a total dose of 1.0 unit of International Standard B† erythropoietin in 3 divided doses on the fifth post-hypoxia day. Each mouse was injected with 0.5 μc Fe^{59} citrate on the 7th post-hypoxia day, exsanguinated 3 days later and Fe^{59} incorporation in RBC determined. Microhematocrits were determined with heparinized capillary tubes. The procedure of analysis of variance(5) as well as the technique of Dunnett(6,7) for comparing several treatments with a single control were used in the statistical analysis.

Results. Table I shows the initial and final renal blood flows and hematocrits in dogs following several degrees of renal artery constriction. All mean 96 hour RBF values were less than that of their respective initial values following constriction (+15 min) or sham operation. The decrease in RBF could have been due to the total blood withdrawn (ap-

* Supported by USPHS Grant AM-02973.

† International Standard B Erythropoietin was obtained from Bureau of Standards, Nat. Inst. For Med. Research, London, England.

TABLE I. Renal Artery Blood Flow and Hematocrit Values in Dogs* Following Renal Artery Constriction.

Group	Body wt (kg)	Kidney wt (g)	Hematocrit (%)		Renal artery blood flow‡				
					ml/g/min†			% Preconstriction control	
			0 hr	96 hr	0	+15 min	96 hr	+15 min	96 hr
A	10.8 ± .28	39.0 ± .04	43.5 ± .31	38.3 ± .62	2.57 ± .03	—	2.33 ± .02	100.0	91.0
B	13.0 ± 1.5	42.0 ± 4.5	39.3 ± 2.9	40.3 ± 3.4	2.79 ± 2.3	1.98 ± .29	1.57 ± .52	71.0	57.5
C	12.9 ± .55	45.3 ± 3.6	40.5 ± 1.1	35.3 ± 2.3	2.77 ± .43	1.39 ± .21	1.20 ± .17	50.0	43.5
D	10.4 ± 1.32	30.5 ± 6.8	37.7 ± 3.4	27.3 ± 1.45	3.88 ± .65	1.37 ± .52	.87 ± .20	35.4	22.4

± = Standard error of mean.
* Each value represents the mean for 4 dogs in each group.
† Renal artery blood flow expressed in ml/g final kidney weight per min.
‡ Time after initial (0) placement of clamp.

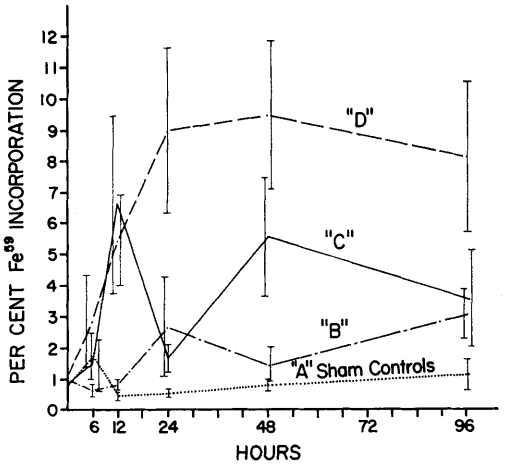

FIG. 1. Mean 72 hr Fe^{59} incorporation in RBC values in polycythemic mice injected with plasma from dogs following renal artery constriction or sham operation. (A) - sham controls - 91-100% preconstriction flow values, (B) - 57.5-71% preconstriction flow values, (C) - 43.5-50% initial flow and (D) - 22.4-35.4% initial flow. Each point represents the mean value for 4 dogs (5-7 mice per plasma) with standard error of means. The mean Fe^{59} incorporation in RBC values for the saline control mice (30 mice) and mice receiving 1.0 unit International Standard B (64 mice) in the dog plasma erythropoietin assays were .70±.07 and 11.85±.80 respectively.

prox. 350 ml) over the 96 hour period for the erythropoietin determinations. With the exception of the 57.5-71% flow group, the final mean hematocrit value was less than that of the initial value in all groups.

As demonstrated in Fig. 1, when renal blood flow was reduced to a level of 22.4-35.4% and 43.5-50% of the preconstriction value a prompt rise in plasma levels of erythropoietin was seen. ESF titers were significantly ($P < .025$) elevated, when compared with the preconstriction plasma levels of ESF, as early as 12 hours in the groups (C and D) following initial constrictions to either 35.4% or 50% of initial flow. We have estimated from our International Standard B dose-response curve the number of units of ESF in the plasma samples following constriction to 22.4-35.4% of initial renal blood flow. ESF titers in this group were approx. .033 units/ml at 12 hours and .17 units/ml at 24 hours. None of the initial plasma samples contained sufficient ESF to be measured on our D-R curve. A slight but not significant increase in plasma

ESF levels was seen 24 hours following reduction to 57.5-71% preconstriction flow levels. The sham operated dogs did not show a significant elevation in plasma erythropoietin titers at any time during the 96 hour period. The ESF titers in the group initially constricted to 35.4% of the preconstriction value were significantly ($P<.05$) higher at 24 hours than that of the group constricted to 50% initially. Plasma levels of ESF in the group initially constricted to 50% were significantly ($P<.01$) higher at 12 hours than that of the group initially constricted to 71%. Therefore, in general the greater the degree of constriction, the more marked the stimulus of ESF production.

Kidneys removed after 96 hours constriction to a range of 35.4-22.4% of initial flow (Group D) showed varying degrees of cloudy swelling. None of the kidneys removed 96 hours after sham operation (A) or reduction initially to 71% or 50% blood flow (B & C) showed any degenerative change.

Discussion. The present studies demonstrate a relationship between renal blood flow and erythropoietin production. A significant increase in plasma levels of ESF was seen with an initial reduction of as little as 29% (71% of initial flow) in renal blood flow. However, the most marked increase in ESF elaboration was seen when RBF was reduced to between 22 and 35% of initial flow or below 1.0 ml/g/min. These findings may be correlated with the unique relationship between renal blood flow and extraction of oxygen by the kidney from renal blood. In contrast to other organs, a moderate reduction in renal blood flow results in a decrease in renal uptake of oxygen. Thaysen *et al*(8) have concluded that as the glomerular filtration rate (GFR) decreases the sodium reabsorption, and hence the work of the kidney, is also decreased. When the renal blood flow falls to 1.0 ml/g/min the pressure in the glomerular capillaries is insufficient for glomerular filtration to occur. The kidney is very similar to other organs when glomerular filtration ceases in that more oxygen is extracted per ml of blood with further reduction in renal blood flow. Oxygen consumption of the kidney was reported(8) to be approximately 1.0 μmol/g/min when RBF is reduced to this level and was considered to represent the basal oxygen uptake of the kidney. Therefore, reduction in RBF below 1.0 ml/g/min probably produces sufficient renal hypoxia to stimulate ESF production. However, prior to postulating that renal blood flow must be reduced below this level before sufficient renal hypoxia is produced to result in the increase in ESF production, some consideration must be given to the slight to moderate increase in ESF levels seen in dogs where renal blood flow has been reduced only to slightly less than 2.0 ml/g/min (43-50 and 57-71% flow groups). It is possible that a more marked reduction in blood flow occurs in some critical renal vascular beds than is reflected in the total renal blood flow measurement. Such a shunting mechanism was proposed several years ago by Trueta *et al* (9). If blood is shunted away from certain cortical glomeruli, sufficient hypoxia to stimulate erythropoietin production may occur in these structures at total renal blood flows greater than 1.0 ml/g/min.

It is of interest that following the initial rise the time course of the elevation of erythropoietin levels in plasma was sustained throughout the period of constriction in all groups with the exception of a drop at 24 hours in the group constricted to a range of 43-50% of initial flow. Other erythropoietic stimuli such as a single massive bleeding or a large dose of cobalt produce a maximum titer of erythropoietin in plasma at about 8 and 12 hours respectively and show a gradual decline reaching control levels after 70 hours (10). The erythropoietic effects of a single stimulus, such as cobalt or bleeding, are only transient and diminish as the stimulus is removed because of the disappearance of cobalt from plasma and the operation of a compensatory mechanism for replenishing the blood following the bleeding stimulus. The sustained rise in erythropoietin in our studies following the peak is probably due to the constancy of the stimulus which is being maintained throughout the 96 hour period. As indicated by the lower renal blood flow at the end of each experiment the degree of renal artery constriction and reduction in renal

blood flow probably become progressively greater throughout the experiment. If renal artery constriction stimulates ESF production through a renal hypoxic mechanism, the sustained ESF activity in plasma while constriction is maintained would indicate that the kidney is capable of enhanced synthesis of ESF, for at least 4 days, when the hypoxic stimulus is continued.

Reduction in renal blood flow due to direct constriction of the renal artery by other factors also results in an increase in plasma ESF. Angiotensin has been found to produce a decrease in RBF and an increase in plasma ESF titers(11). It is also of interest that Piliego *et al*(12) have reported elevated plasma levels of ESF in humans following venous stasis of the lower limbs and have suggested reduction in renal artery blood flow as the cause. An association between erythrocytosis and renal artery stenosis has also been reported by Frohlich *et al*(13). It is possible that renal hypoxia resulting from the decrease in renal blood flow elevates the level of an erythropoietin producing factor in the kidney. This renal factor is postulated to activate a precursor of erythropoietin in plasma(14,15).

Summary. Reduction in renal artery blood flow in dogs with the use of a modified Goldblatt clamp to a range of 22.4-35.4% of preconstriction values for 96 hours resulted in a significant increase in plasma levels of erythropoietin at 12 hours. When renal blood flow was reduced to a level 43.5-50% of initial flow erythropoietin titers were also elevated at 12 hours, whereas, reduction to a range of 57.5-71% of initial values only resulted in a slight rise in erythropoietin levels at 24 hours. The mechanism of enhanced erythropoietin elaboration following reduction of renal blood flow is postulated to be the result of renal hypoxia.

The authors gratefully acknowledge the technical assistance of Mrs. Judy Parker, Mr. Carroll Osburn and Miss Jayne Cox and are also indebted to Dr. Y. C. Kim, Pathology Department, Baptist Memorial Hospital, Memphis, for his histological evaluation of the kidneys.

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Received January 27, 1967. P.S.E.B.M., 1967, v125.