The Effect of Cobalt on Erythropoietin and Kininogen Levels in Rat Plasma (36896)

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The kinins (*e.g.*, bradykinin) are tissue hormones, believed responsible for local effects on vascular smooth muscle. Cobalt may cause tissue anoxia (1, 6, 11); and such anoxia could trigger changes in the vascular bore possibly mediated by the kinins. The kinins are also believed involved in inflammatory processes and hemorrhagic shock (16).

The kinins are formed from kininogen, a circulating serum protein. Changes in kininogen levels could indicate alterations in the rate of kinin production or changes in the rate of kininogen synthesis. Increased kinin formation at tissue sites could presumably reduce the concentration of kininogen in the plasma, especially if the rate of kininogen synthesis is slower than kininogen utilization.

Erythropoietin (ESF) may be formed by the interaction of a renal erythropoietic factor (erythrogenin, REF) and a serum protein (2, 3). This serum erythrogenin substrate has not been characterized, but erythropoietin is believed to be an $alpha_1$, $alpha_2$-globulin, as it is present in the serum (8–10, 12, 15). Since the kinins are formed from a serum alpha globulin substrate (kininogen (17), it is possible that this large heterogenous serum protein group could serve as a substrate from which both the kinins and erythropoietin are produced. Cobalt stimulates the *in vivo* production of erythropoietin (5). Changes in kininogen levels were compared with the appearance and disappearance of erythropoietin after cobalt treatment.

Materials and Methods. Determination of kininogen in cobalt treated rats. Male Sprague-Dawley rats (300–350 g) were given a subcutaneous injection of cobaltous chloride (1.0 ml containing 75 μmoles). The rats were divided into 5 groups, 5 rats/group. One group (untreated) served as a control, and the other groups were sacrificed 4, 8, 12 and 24 hr after treatment with cobaltous chloride. The animals were bled by aortic puncture. Heparin (157 units) was added to the whole blood which was centrifuged; and the plasma obtained was frozen in plastic containers. Care was taken to use only plastic vessels and syringes since contact with glass can activate kinin formation (7).

In order to measure kininogen, the plasma precursor of kinins, the kininogen was quantitatively converted to kinin by the procedure of Diniz *et al.* (4). The kinin concentration was measured by the rat uterus bioassay. This bioassay consists of one uterine horn suspended in a bath containing 10 ml of a solution containing NaCl, 9.0 g; KCl, 0.30 g; $CaCl_2 \cdot 2H_2O$, 0.25 g; $NaHCO_3$, 0.20 g; $MgCl_2 \cdot 6H_2O$, 0.20 g; atropine sulfate, 1.0 mg/liter of glass distilled water. A Narco isotonic myograph was employed in this assay and the contractions were recorded on a Narco physiograph model DMP-4A.

The height of the muscle twitch in the uterus assay is directly proportional to kinin levels in the dose range studied. Kinin levels are directly proportional to the kininogen content in this procedure. A standard log dose–response curve with known concentrations of bradykinin was established for each bioassay.

Polycythemic mouse erythropoietin assay. Female Carworth (CF-1) mice, 20–25 g in weight, were used for this assay. The mice were kept in a hypobaric chamber at a pressure of 0.5 atm for a period of 3 wk. They were taken from the chamber once a day for a period of 1 hr for food and water. At the end of this period of 3 wk in the chamber, the mice were removed and allowed to equilibrate at 1 atm for 2 days. These mice were polycythemic as indicated by an elevated hematocrit (mean above 60%). The erythropoietin

TABLE I. Relative Quantity of Kininogen and Erythropoietin in the Plasma of Cobalt Treated Rats.[a]

Time after cobalt treatment (hr)	Plasma kininogen level[b] [twitch height of rat uterus (mm)]	% ^{59}Fe Inc/ml plasma[c]
—	—	Saline control[d]– 1.28 ± 0.07
Untreated rats	17.0 ± 0.13	1.38 ± 0.16
4	31.0 ± 0.21[e]	2.32 ± 0.04
8	19.0 ± 0.17	5.14 ± 0.43
12	14.0 ± 0.09	9.72 ± 0.08
24	32.0 ± 0.24[e]	5.28 ± 0.08

[a] A significant increase ($p < 0.05$) in the ^{59}Fe of assay mice was obtained in all groups receiving plasma from cobalt treated rats compared to assay mice receiving plasma from untreated rats.

[b] The height of the uterine twitch is directly proportional to the kinin level in the dose range employed. The kinin level is directly proportional to the kininogen content of the plasma since kininogen is quantitatively converted to kinin by the procedure of Diniz *et al.* (4). The kinin content of the plasma is therefore an indicator of the kininogen level in this procedure.

[c] Each value represents the mean ± SEM of 15 assay mice.

[d] Assay mice receiving only saline.

[e] Significantly different from kininogen in the untreated, 8 and 12 hr cobalt treated groups at $p < .05$.

assay took a period of 5 days to complete. On Day 1, the mice were divided into treatment groups and received an intraperitoneal injection of the substance to be tested. One group of mice received physiological saline as a control. On Day 3, all mice received 250,000 cpm of ^{59}Fe (0.1 ml) via the tail vein. On Day 5, the mice were bled by cardiac puncture and 0.5 ml of blood was withdrawn from each mouse. Blood samples were counted in a Packard Tri-Carb scintillation spectrometer, Model 3375, equipped with an Auto-Gamma spectrometer.

Results. The plasma from the 4 hr cobalt treated group showed a significant ($p < .05$) increase in kininogen concentration over that of the untreated group (Table I). The kininogen level dropped in the 8 hr cobalt treated group and continued to decrease in the 12 hr group when compared to the kininogen level of the 4 hr group. At 24 hr after cobalt treatment, the kininogen concentration increased over that of the 8, 12 hr treatment groups and the untreated group; and was as high as the kininogen level of the 4 hr cobalt treatment group.

The erythropoietin content of the rat plasma was determined by measuring the stimulatory effect of this plasma on the percentage ^{59}Fe incorporation into the erythrocytes of polycythemic mice. The plasma from all cobalt treated groups significantly increased the percentage ^{59}Fe incorporation of assay mice ($p < .05$), as compared to the assay group receiving plasma from untreated rats. The 12 hr cobalt treatment group had the highest percentage ^{59}Fe incorporation, indicating the time of maximum erythropoietin production. This trend follows that of Goldwasser (5).

Discussion. Bradykinin is a 9-amino acid polypeptide produced by the action of an enzyme kallikrein on an alpha$_2$-globulin (kininogen) in the plasma (17). Roche e Silva (13) suggested the name "kinin hormones" for this class of polypeptides which have no specialized gland of secretion, being released from inactive precursors (kininogens) in the plasma and most tissues including the intestinal tract and the central nervous system.

The kininogen level is significantly affected by cobalt treatment, as is the erythropoietin level of rat plasma (Table I). Of interest is the possibility of a relationship between kininogen level in the plasma and erythropoietin production. The erythropoietin content of the plasma rises significantly ($p < .05$) at 4, 8 and 12 hr, reaching maximum at 12 hr after cobalt treatment and decreasing at 24 hr. The kininogen level of the plasma rises abruptly at 4 hr after cobalt treatment and returns to approximately normal levels at 8 hr. The increase in the kininogen level of the plasma appears to precede the rise in erythropoietin level. Such behavior would be expected if the kininogen serves as a substrate or precursor for the production of erythropoietin. If this is the case, the second rise in kininogen level of the plasma (24 hr) could indicate a secondary rise in erythropoietin activity in the plasma, which should

occur sometime after 24 hr. The period from 24 to 50 hr after cobalt treatment does appear to be a time when the erythropoietin level in the plasma levels is sustained before declining to normal values (5). Erythropoietin is believed to be formed by the interaction of the renal erythropoietic factor (REF, erythrogenin) with a serum protein substrate (2, 3). The alpha globulins, of which kininogen is a part, may serve as a substrate for the renal erythropoietic factor. Cobalt may produce a sustained (lasting at least 24 hr) increase in the plasma kininogen level which is not apparent because of the high rate of erythropoietin production occurring between 4 and 12 hr after cobalt treatment. The high rate of erythropoietin production may reduce the circulating kininogen level, if kininogen can serve as an erythropoietin precursor. As the rate of erythropoietin production declines, the kininogen level would then be expected to rise again to the high level it attained within 4 hr after cobalt treatment. Erythropoietin could be also formed from other proteins in the serum which share some structural similarity to kininogen, but which do not serve as a precursor of the kinins.

It is equally possible that the alterations in kininogen level after cobalt treatment may not be related to changes in the erythropoietin content of the plasma. Tissue anoxia, believed to be initiated by cobalt treatment, could stimulate kininogen synthesis or decrease its utilization possibly by reducing the rate of conversion of kininogen to kinin. Such alterations could result in the increase in kininogen levels noted at 4 and 24 hr after cobalt treatment.

Summary. Kininogen and erythropoietin are both alpha glycoproteins. Erythropoietin activity increases in the plasma of rats treated with cobalt, reaching a maximum activity 12 hr after cobalt treatment. The kininogen level of the plasma rises significantly after cobalt treatment, reaching maximum 4 hr after cobalt treatment. It appears that an increase in the kininogen level of the plasma precedes the increase in the erythropoietin activity of the plasma. This could suggest that the alpha globulins, of which the kininogen is a part, may be serving as a substrate for the production of erythropoietin.

The data could also suggest that tissue anoxia caused by cobalt treatment, or cobalt itself, may alter the rate of synthesis of kininogen or the rate of utilization of circulating kininogen.

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