Effects of Posterior Hypothalamic Stimulation on Reticulocyte Release and Bone Marrow Microcirculation[1] (37453)

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It has been demonstrated by Linke *et al.* (1) that marked changes in blood flow through the bone marrow occur after hypothalamic stimulation. They reported that sympathetic stimulation reduces blood flow in the bone marrow while increased parasympathetic activity increases blood flow. Electrical stimulation of the posterior hypothalamus also has been reported to produce a slight increase in hematocrit and a significant increase in the percentage of reticulocytes in the peripheral blood of rabbits (2), rats (3), and monkeys (4, 5). These responses apparently are not due to increased circulating levels of ESF since detectable levels of erythropoietin (ESF) rarely have been found. Halvorsen *et al.* (6) reported that only 6 of 16 rabbits had slightly increased ESF levels following posterior hypothalamic stimulation. Mirand *et al.* (4) reported an increased level of ESF in only 1 of 4 monkeys after 8 hr stimulation of the preoptic nucleus.

In an attempt to clarify the role of the hypothalamus and the parasympathetic nervous system in the control of erythropoiesis, the reticulocyte response to posterior hypothalamic stimulation in normal and atropine treated rabbits was determined. In order to investigate further the mechanism of this response, posterior hypothalami of rabbits were stimulated continuously and reticulocyte and hematocrit values determined while the microvascular responses in the bone marrow to hypothalamic stimulation were examined using the *in vivo* microscopic method of McCuskey *et al.* (7).

Materials and Methods. Male or female New Zealand white rabbits, weighing between 3.0 and 3.6 kg were used in the hypothalamic stimulation studies. The experimental protocol was divided into four 14 day periods as follows: (*1*) control period; (*2*) sham-operated period; (*3*) hypothalamic stimulation period and (*4*) hypothalamic stimulation combined with daily injections of atropine (5 mg/kg/day ip). Three rabbits were subjected to hypothalamic stimulation only. Thus, each animal served as its own control. Each period consisted of 6 hr/day for 14 days in a rabbit restraining cage. After the control period, insulated bipolar electrodes were implanted bilaterally in the posterior hypothalamus under pentobarbital anesthesia (30 mg/kg) according to the stereotaxic coordinates described by Sawyer *et al.* (8). The stimulation parameters were those used by Halvorsen *et al.* (6). The localization of the electrodes in the hypothalamic region was confirmed in some rabbits with the use of histological sections of the brains. Reticulocytes were counted before and on Days 3, 5, 7, 10, and 14 of each study. Depending upon the reticulocyte level, blood was withdrawn from the central artery of the ear for ESF assay in exhypoxic polycythemic mice (9).

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TABLE I. Reticulocyte Counts in Rabbits Before and After Posterior Hypothalamic Stimulation.

Days	1	3	5	7	10	14
Control period (6)	1.43 ± 0.32	1.45 ± 0.32	1.43 ± 0.18	1.38 ± 0.21	1.36 ± 0.21	1.38 ± 0.24
Sham-operated period (6)	1.95 ± 0.31	1.60 ± 0.19	1.50 ± 0.09	1.45 ± 0.10	1.43 ± 0.11	1.46 ± 0.09
Stimulation period (9)	2.41 ± 0.59	4.98 ± 1.09	5.93 ± 1.10[a]	5.96 ± 0.83[a]	6.06 ± 0.62[a]	6.49 ± 0.63[a]
Stimulation plus atropine (5 mg/kg/day) period (6)	1.46 ± 0.20	1.26 ± 0.20	1.28 ± 0.18	1.48 ± 0.30	1.40 ± 0.25	1.43 ± 0.25

[a] $p < 0.05$ when compared to control, sham-operated or stimulation plus atropine. Numbers in parenthesis represents the number of rabbits in each group.

In another series of experiments, seven male or female New Zealand albino rabbits weighing between 3.0 and 3.6 kg were used to study the effects of posterior hypothalamic stimulation for 24 hr on the hematocrit and reticulocyte counts. The response of the microvascular system of the tibial marrow to posterior hypothalamic stimulation was studied in 3 of these rabbits containing a titial bone marrow chamber (7) installed 3 to 6 months previously. One week following the implantation of electrodes (as described above) the animals were stimulated for 24 hr using the following parameters: 50 cps, 1 msec duration, and 3–5 V. An interval timer was introduced into the system in order to deliver 30 seconds of stimulation followed by 30 sec rest. Peripheral reticulocytes were counted prior to and following 24 hr stimulation using the New Methylene Blue technique. Hematocrits were determined concomitantly. Sham-operated animals served as controls. Each of the 3 animals used in this series were studied for one 24-hr period only.

Post-hypoxic polycythemic mice prepared according to a modification of the method of Cotes and Bangham (9) were used to assay erythropoietin. HAM/ICR strain female mice (22–25 g) were made polycythemic by exposure to 0.42 atmosphere for two weeks. The mice were injected subcutaneously with ½ the total dose of either saline, IRP Standard erythropoietin[5] or 0.5 ml of rabbit plasma on the fourth and fifth days following their removal from the hypobaric chamber. Each mouse received 0.5 μCi of radioactive iron (^{59}Fe) on the sixth post-hypoxic day via the tail vein. Two days later (8th post-hypoxic day) each animal was exsanguinated via cardiac puncture, the blood sample counted in a Packard Scintillation counter and percent of ^{59}Fe incorporation into red blood cells determined. The data were analyzed using the technique of analysis-of-variance (10). If differences in group means were found, the Dunnett's multiple comparisons technique for comparing several treatments with a single control was used for the statistical analysis.

Results. 1. Effects of atropine on the reticulocytosis induced by hypothalamic stimulation. Six control rabbits (nonstimulated) were placed in a restraining cage 6 hr each day for 14 days. No change in the reticulocyte levels or hematocrit were observed (Table I). These rabbits with electrodes in the posterior hypothalamus were placed in a cage 6 hr per day for 14 days but were not stimulated. Again no changes in hematocrit or reticulocyte counts were observed in this group of rabbits (Table I). Nine rabbits were stimulated 6 hr each day for 14 days. A sustained and marked reticulocytosis as well as an increase in hematocrit were observed. The reticulocytosis seen was significantly ($p <$ 0.05) increased after the fifth day of stimulation (Table I). When plasmas from the stimulated rabbits were assayed in post-hypoxic polycythemic mice, an increase in ^{59}Fe incorporation in red blood cells was observed in the polycythemic mice receiving plasma from only one rabbit (0.07 IRP units/ml).

[5] Supplied by the Bureau of Standards, National Institute of Medical Research, Mill Hill, London, England.

No significant increase above the saline controls was seen in polycythemic mice receiving plasmas from eight additional stimulated rabbits.

Pretreatment with atropine (5 mg/kg/day) in six rabbits prior to hypothalamic stimulation blocked completely the reticulocyte response (Table I) as well as the changes in hematocrit. The inhibitory effect of atropine in the 6 rabbits studied was manifested after three days of stimulation and was sustained throughout the 14-day period.

2. Effects of hypothalamic stimulation on reticulocyte values and bone marrow microcirculation. The results of the 24 hr stimulation showed the following: posterior hypothalamic stimulation for a 24-hr period produced a significant ($p < 0.001$) increase in reticulocyte values. The reticulocyte counts increased from 1.52 ± 0.30 to 3.44 ± 0.31% after 24 hr continuous stimulation (Table II). The hematocrit increased from a mean of 40.8 to 43.0% during the same time period but this change was not statistically significant.

Figure 1 shows the same microscopic field of the bone marrow at the beginning of one experiment, after 1 and 24 hr of continuous posterior hypothalamic stimulation. The initial response in the bone marrow after 1 hr stimulation was vasoconstriction and reduced blood flow. The mean decrease in the number of vessels with visible blood flow was 20.6% and the mean decrease in vessel diameter was 28%. However, after 24 hr of continuous stimulation, the number of vessels with visible blood flow had increased to 38.2% above control values; the mean diameter of these vessels was increased by 37.7% with a concomitant increase in blood flow when compared to that of controls (Fig. 2).

Discussion. The posterior hypothalamus has been postulated to play a role in ESF production. Electrical stimulation (3–6, 9, 12–16) as well as injury of the hypothalamic complex (17) have been reported to induce increases in peripheral red cells, reticulocytes and hemoglobin. Halvorsen *et al.* (6) and Seip *et al.* (16) have reported that electrical stimulation of the hypothalamus in rabbits increased reticulocytes in peripheral blood as well as an increase in the red cell mass. Halvorsen (17), in electrocoagulation studies of the anterior, median and posterior hypothalamus, found that posterior hypothalamic lesions in rabbits prevented the increase in ESF levels usually seen after exposure to hypoxia.

TABLE II. Reticulocyte Values Before and After 24 hr Continuous Stimulation of Posterior Hypothalamus.

Animal	Initial	24 hr
1	2.6	2.8
2	2.5	3.3
3	1.4	3.5
4	1.2	4.4
5	1.0	2.0
6	0.6	3.9
7	1.4	4.2
Mean ± SE	1.52 ± 0.30	3.44 ± 0.31[a]

[a] Indicates $p < 0.001$ when compared with the initial mean value.

Our present findings confirm that posterior hypothalamic stimulation produces a significant increase in reticulocytes in peripheral blood. In addition, atropine also was found to block the increase in blood reticulocytes following hypothalamic stimulation. We also have found (18) that rabbits exposed to hypoxia and pretreated with atropine show significantly less ESF production in response to hypoxia when compared to that of the hypoxic controls. These studies indicate that a cholinergic mechanism is involved in the erythropoietic response to hypoxia (18) and to hypothalamic stimulation (3, 14). Other studies which support the role of a cholinergic mechanism in erythropoiesis is the report by Davis (19) who found that the administration of 200 units cholinesterase for 5–7 days increased blood reticulocytes in rats.

Halvorsen *et al.* (6) demonstrated in rabbits that renal blood flow decreases slightly during posterior hypothalamic stimulation, although they could not correlate the decrease in renal blood flow with an increase in plasma ESF levels. On the other hand, Finne and Skoglund (20) have reported that the increase in ESF titers following hypoxia are essentially the same in intact rats and

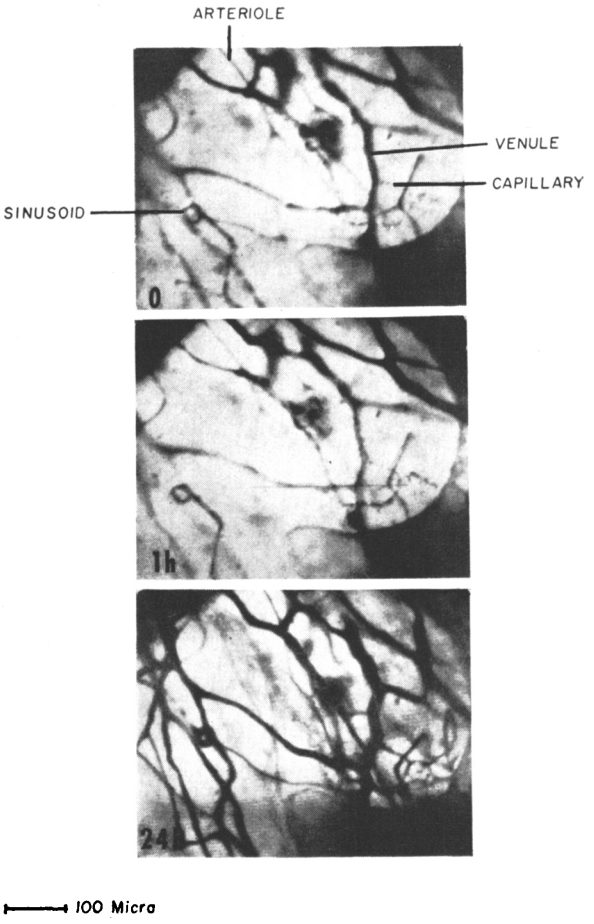

FIG. 1. Photomicrographs of the same microscopic field of rabbit tibial bone marrow initially (0 hr) and after 1 and 24 hr posterior hypothalamic stimulation.

rats with denervated kidneys. They pointed out that the reduction in total blood flow is not a primary mechanism in the increased ESF production following hypoxia.

Halvorsen *et al.* (6) also have shown that blood reticulocytes can increase without a concomitant increase in ESF. They suggested that the autonomic nervous system alone could mediate the release of reticulocytes and consequently that the reticulocyte levels could increase without a simultaneous increase in ESF. If both stimuli are present they were postulated to act synergistically.

On the other hand, posterior hypothalamic stimulation produces a significant increase in reticulocytes in peripheral blood within 24 hr. This is accompanied by a significant increase in blood flow through the microvasculature of the bone marrow. It is not known whether similar changes in flow occur in other vascular beds. However, similar changes are probable considering the diffuse affects of hypothalamic stimulation. These results suggest the existence of a neurovascular mechanism for the rapid release of stored reticulocytes from the sinusoids of the bone marrow. Such an "autotransfusion" mechanism would be consistent with the rapid shifts of circulating red cell mass following exercise or stress. It is not known whether this mechanism is involved in the day-to-day control of erythropoiesis. These studies do not rule out the possibility that increased ESF levels may be seen after more prolonged hypothalamic stimulation. Further studies may reveal that atropine is capable of blocking the response

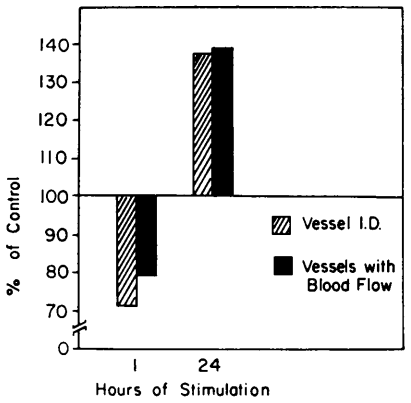

FIG. 2. Represents the mean % change in the internal diameter (i.d.) of the tibial bone marrow vessels and the mean % change in the number of vessels with active blood flow. The number of vessels in 5 microscopic fields chosen at random were counted in each rabbit. The number of vessels counted in each microscopic field varied from 7 to 21 vessels/field.

of the marrow microvasculature to hypothalamic stimulation.

Summary. Posterior hypothalamic stimulation in rabbits was found to produce a significant increase in blood reticulocytes as well as a slight increase in hematocrit. The increase in reticulocytes and hematocrit following hypothalamic stimulation were not seen during the control or sham-operated periods. Atropine was found to block the reticulocyte response to hypothalamic stimulation.

During 24 hr continuous posterior hypothalamic stimulation vasoconstriction and a reduced number of vessels with blood flow in the bone marrow microvasculature were seen during the first hour of stimulation, while by 24 hr vasodilatation and an increase in the number of vessels with blood flow was observed. These changes in the bone marrow circulation were associated with a significant increase in blood reticulocytes during hypothalamic stimulation. These studies suggest the existence of a neurovascular mechanism for the rapid release of stored reticulocytes from marrow sinusoids of the bone marrow.

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