Effect of Hypothalamic Lesions on Erythropoietin Production in Weanling and Adult Rats

Recent investigations[1,2] indicate that the hypothalamus may participate in the regulation of erythropoiesis in rabbits, while work in the rat shows that long-term hypoxia has no effect on the erythropoietic response of lesioned rats[3]. The present communication reports on the ability of weanling and adult rats with hypothalamic lesions to produce erythropoietin under hypoxic hypoxia.

Weanling male Sprague-Dawley rats, weighing between 66 and 83 g, received bilateral electrolytic lesions in the supraoptic, ventromedial, dorsomedial and mammillary hypothalamic nuclei. Sham-operated rats served as controls (Table I). Adult rats of the same strain and sex but weighing between 268 and 337 g were grouped and treated similarly (Table II). The hypothalamic lesions were placed with a stereotaxic instrument (Baltimore Instrument Co.), using a spar varnish-coated stainless steel electrode of 0.25 mm diameter, from the bared tip of which an anodal current of 1.5 mA was allowed to flow for 10 sec. The coordinates for the placement of the lesions had been established previously[4]. The sham-operated animals were treated similarly, except for the flow of the electrical current. The rats were accommodated in single cages in a room kept at 74 °F and given tap water and lab chow ad libitum. 2 weeks following the hypothalamic operation they were subjected to low oxygen atmosphere (10%) for 36 h, after which they were anesthetized with ether, exsanguinated and the plasma collected for assay of erythropoietin in hypertransfused polycythemic mice[5]. The brains were treated in the usual manner[6] and the localization of the hypothalamic lesions was determined according to the atlas of DE GROOT[7].

(A) *Weanling rats.* Table I shows that weanling rats with lesions in the dorsomedial and mammillary hypothalamic nuclei did not produce detectable amounts of erythropoietin under hypoxia whereas rats with lesions in the supraoptic and ventromedial nuclei did.

(B) *Adult rats.* Table II indicates that adult rats with lesions in the mammillary nuclei did not produce erythropoietin under hypoxic hypoxia. Unlike the weanling rats, however, adult animals with lesions in the supraoptic and ventromedial nuclei showed some decrease in their ability to produce erythropoietin in low oxygen atmosphere.

Studies on the role of the central nervous system in the regulation of erythropoiesis are difficult to interpret since stimulation or destruction of hypothalamic areas may elicit both autonomic-nervous or neuroendocrine responses. Thus, the hypothalamus may affect erythropoiesis via releasing factors for pituitary trophic hormone secretion, i.e. testosterone via LH, thyroid hormone(s) via TSH and adrenocorticotrophic hormone via CRF[8]. The above hormones are known to influence indirectly erythropoiesis. Second, the hypothalamus may do so via the autonomic system by affecting cardiopulmonary changes producing alterations in blood flow which in turn may induce the kidney or other erythropoietin-producing organs to release or block erythropoietin[5].

It is noteworthy that there appears to be an age difference in the erythropoietic response to hypothalamic lesions. This may well be due to a difference in representation of function in a particular neuronal assembly in the rat hypothalamus at different ages, or to a different sensitivity depending on age. Thus, it has been shown[9] that ventromedial hypothalamic lesions placed in weanling rats result in a greater growth reduction than placement of such lesions in older rats. A similar pattern has been demonstrated for the systolic blood pressure[10].

The present data demonstrate that the rat, like the rabbit[1] and monkey[11], shows changes in erythropoietin levels following stimulation or the placement of lesions in the hypothalamus. FELDMAN et al.[12], in studying the effects of central nervous system stimulation in rats with chronically implanted electrodes, were able to observe erythropoiesis but were not able to detect erythropoietin in the plasma of such rats. To resolve any differences in the results obtained from different laboratories on the role of the hypothalamus in erythropoiesis and erythro-

Table I. Effects of low oxygen atmosphere (10%) upon erythropoietin levels in hypothalamic-lesioned and sham-operated young rats

Pooled plasmas from rats with lesions at the site of:	% 24 h blood Fe^{59} uptake in polycythemic mice
(A) (1) Supraoptic nucleus[a]	4.03 ± 1.5[b]
(2) Ventromedial nucleus	3.05 ± 1.5
(3) Dorsomedial nucleus	0.77
(4) Mammillary nucleus	0.50 ± 0.20
(B) Sham controls	1.45 ± 0.72

[a] Approximately in the vicinity. [b] ± standard deviation.

Table II. Effects of low oxygen atmosphere (10%) upon plasma erythropoietin levels in hypothalamic-lesioned and sham-operated adult rats

Pooled plasmas from rats with lesions at the site of:	% 24 h blood Fe^{59} uptake in polycythemic mice
(A) (1) Supraoptic nucleus	1.18 ± 0.23[b]
(2) Supraoptic nucleus[a]	0.50 ± 0.13
(3) Ventromedial nucleus	1.80 ± 0.39
(4) Ventromedial nucleus[a]	1.01 ± 0.04
(5) Mammillary nucleus	0.64 ± 0.12
(6) Mammillary nucleus[a]	0.18 ± 0.09
(B) Sham controls	4.14 ± 0.55
(C) Non-sham controls	3.98 ± 0.24

[a] Approximately in the vicinity. [b] ± standard deviation.

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poietin production, it appears important that the length of stimulation, the size and place of the lesion, age difference, species, etc. be as nearly comparable as possible before any ultimate evaluation can be made[13,14].

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Zusammenfassung. Es wurde die Wirkung von künstlichen, bilateralen, elektrolytisch gesetzten Läsionen in verschiedenen Bezirken des Hypothalamus auf die Bildung von Erythropoietin untersucht: Jugendliche (entwöhnte) Ratten reagieren nicht gleich wie erwachsene Ratten, und die verschiedenen Kerne des Hypothalamus beeinflussen die Erythropoietin-Bildung verschieden.

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Variation of the Fine Structure in Granulocytes of Great Apes

As part of a continuous study on comparative primate hematology, the leucocyte ultrastructure of the Great Apes was investigated with the electron microscope. The studies were performed on peripheral blood obtained from chimpanzees, gorillas and orangutans. Investigations by light microscopy have shown that white cell morphology of the Great Apes is very similar to, and almost indistinguishable from, morphology of human leucocytes, although certain typical morphological characteristics have been found[1]. On the other hand, different histochemical properties have been demonstrated in neutrophilic leucocytes of man, gorilla, chimpanzee and orangutan[2]. The present study showed the presence of characteristic differences in the ultrastructure of gorilla and orangutan granulocytes.

Blood was obtained from 3 animals of each species (*Gorilla gorilla, Pan troglodytes, Pongo pygmaeus*) from the femoral vein by free flow through plastic tubing into a heparinized, siliconized centrifuge tube. Cells were prepared for electron microscopic study by routine methods[3] with some modifications. Osmium tetroxide or glutaraldehyde were used for fixation and sections were stained in saturated uranyl acetate solution in 20% alcohol and lead citrate. Examination of sections was made in a Siemens Elmiscop I, electron microscope.

At least 1000 cells were examined from each species, and the following number of pictures were taken for further examination: gorilla, 109 pictures from 105 different cells; chimpanzee, 242 pictures from 200 different cells; orangutan, 106 pictures from 95 different cells. The final magnifications varied from × 4000 to × 45,000.

The *neutrophilic* leucocytes from gorilla and chimpanzee were indistinguishable from each other and similar to human neutrophils, as described by Low and FREEMAN[3] and others[4]. The cytoplasm was well filled with specific granules which had a limiting membrane. 2 kinds of granules were consistently present: larger, round or ovoid granules with a very dense content and smaller, elongated rod-like granules of somewhat lower density. The orangutan neutrophils showed the same characteristics. However, in addition to the 2 kinds of specific granules, a third type of granule was consistently present. These granules measured up to 0.7 μ were rod-like or spindle shaped and were surrounded by a membrane. Characteristically, their content was not homogenous; as in the other types of specific granules, but consisted of numerous fibers or needles running parallel to each other from end to end in the long axis of the granules. Occasionally these fibers were densely packed, forming crystal-like densities located centrally in the granule (Figure 1). This type of granulation seems to be a morphological characteristic of the orangutan neutrophil, as it has not been found in neutrophils of other Great Apes, nor has it been found in neutrophils of selected Old World monkeys (Cercopithecideae) or in neutrophils of New World monkeys (Ceboideae)[5]. The granules can also be demonstrated after glutaraldehyde fixation. On morphological grounds alone it can be said that they are distinctly different from the oval azurophilic and the specific elongated granules characterized in neutrophils by previous authors[6–8]. Since these 2 types of granules are equally present in orangutan neutrophils further histochemical studies may reveal additional differences and characteristics.

The *eosinophilic* leucocytes of the orangutan were indistinguishable from human eosinophils. In addition, however, the cells of this species usually showed one or more dark inclusions of irregular shape with lipid-like staining characteristics. In light microscopy, the orangutan eosinophils show characteristic vacuoles if fixed in methanol and stained with Wright-Giemsa stain. These vacuoles are conceivably lipid, and identical or related to the lipid-like bodies found in electron microscopy. These structures are not seen in human eosinophils nor are they commonly present in eosinophils from other primate species. The gorilla eosinophil differs from all other eosinophils in the structure of the specific eosinophilic granules. The matrix of the granules is darker than in eosinophilic granules of

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