Erythropoietic Response to Isomeres of Triiodothyronine

Alteration in thyroid function influences the synthesis and life span of the red blood cell. Anemia is a consequence of thyroidal hypofunction in man and experimental animals, whereas thyroid hormone administration is characterized by erythropoietic acceleration[1,2]. The augmentation in erythropoiesis resulting from the administration of thyroid hormones has been attributed to the calorigenic properties of the hormones and a resultant imbalance between the tissue oxygen need and oxygen supply. This regional hypoxemia would result in enhancement of erythropoiesis. However, it is now generally accepted that the hypoxic stimulus to erythropoiesis is mediated through the augmented production of the erythropoietic hormone, erythropoietin, by the kidney[3]. There is no agreement as to the role which erythropoietin plays in thyroid-induced erythropoietic stimulation[4-7]. In the present study the capacity of the nephrectomized rat, which essentially is unable to elaborate erythropoietin, to respond erythropoietically to the administration of thyroid hormones was assessed. The role which calorigenesis plays in this situation was assessed by determining the erythropoietic response to L-triiodothyronine (LT 3) and its calorigenically inactive isomere, D-triiodothyronine (DT 3) in the nephrectomized rat, and in an in vitro reticulocyte incubation system.

Female Wistar rats weighing 180–200 g were maintained on a regular laboratory animal diet[8] and provided water ad libitum. Rats were given injections of 25 μg DT 3, LT 3[9] or 0.25 ml of 0.9% saline solution 1 and 6 h after bilateral nephrectomy. Erythropoiesis was assessed by: determination of microhematocrits, and measurement of the erythrocyte incorporation of radio-iron 18 h after the i.v. injection of 1 μc ^{59}Fe/1 μg ^{56}Fe citrate (48 h following nephrectomy). Radioiron partition in the spleen, liver and bone marrow was also determined[10].

Additional experiments tested the effect of LT 3 and DT 3 on the in vitro iron incorporation by reticulocytes. Reticulocytosis was produced in donor rats by the administration of a 2.5% solution of acetyl phenylhydrazine in the drinking water on 4 consecutive days followed by a 2 day hiatus and administration for an additional 4 days. At the end of this time the rats were exsanguinated. A 50% red blood cell suspension was prepared by adding one volume of packed washed erythrocytes of known reticulocyte concentration to an equal volume of Krebs-Ringer Phosphate buffer containing glucose and streptomycin. 2 ml of cell suspension were incubated with normal plasma of known iron content. LT 3, DT 3, saline or an erythropoietically active extract of anemic rat plasma (rat erythropoietin) was added to representative incubates. A previous report presents the in vitro procedure in detail[10]. Radioiron uptake of washed erythrocytes was measured in a well counter; the concentration of iron was also determined, and the total iron incorporation per 0.1 ml of reticulocytes was calculated.

In vivo experiments: The results of the 18 h RBC ^{59}Fe incorporation are presented in Table I. Nephrectomy produced a slight reduction in the RBC ^{59}Fe incorporation determined 48 h following nephrectomy. The administration of either LT 3 or DT 3 produced erythropoietic stimulation to the same degree (Table I). These results suggest that the nephrectomized rat which is unable to produce erythropoietin is capable of responding erythropoietically to both the calorigenically active and the calorigenically inactive isomeres of triiodothyronine. The degree of response, however, is not as great as that produced in an intact rat. This difference may indicate that the erythropoietic response to triiodothyronine is mediated, in part, by erythropoietin, or that the toxic products of uremia produce a nonspecific decrease in erythropoietic response. Support for the latter hypothesis is provided by the diminished erythropoietic response of the nephrectomized rat to exogenous erythropoietin[10].

In vitro experiments: The results of iron incorporation into incubated reticulocytes are presented in Table II.

Table I. In vivo experiments

Intact rats	No. rats	% Radioiron incorporation in 18 h HCT	RBC	Marrow	Liver
Saline	6	44.2 ± 2.2[a]	22.3 ± 2.1[a]	22.3 ± 1.8[a]	10.6 ± 2.3[a]
LT 3	8	43.7 ± 3.1	32.1 ± 3.4	18.4 ± 3.0	12.1 ± 1.9
DT 3	7	45.1 ± 1.8	31.6 ± 2.9	19.4 ± 2.1	14.3 ± 4.4
NX rats					
Saline	8	36.5 ± 1.3	18.7 ± 1.9	20.1 ± 3.6	9.8 ± 3.2
LT 3	8	38.0 ± 3.4	26.5 ± 2.3	24.7 ± 4.8	12.3 ± 3.5
DT 3	8	35.4 ± 2.8	23.8 ± 2.9	26.3 ± 3.7	11.3 ± 2.4

[a] ± Standard deviation.

Table II. In vitro reticulocyte iron incorporation

Material added to incubate	μg Fe/1 ml reticulocytes 2 h	4 h	6 h
Saline	0.56	0.79	0.98
	–	0.81	1.01
	–	0.76	1.00
Erythropoietin	0.46	0.70	0.89
(1 unit)	0.32	0.73	0.82
LT 3	1.08	1.99	2.01
(0.1 μg/ml)	1.03	2.03	2.06
	0.99	2.07	2.11
DT 3	1.11	1.97	1.99
(0.1 μg/ml)	1.10	2.10	2.16
	0.96	1.99	2.10

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Reticulocytes continued to incorporate radioiron during the entire incubation period. Addition of exogenous rat erythropoietin to the incubation media did not produce any alteration in reticulocyte iron incorporation. However, the addition of LT 3 or DT 3 in concentrations of 0.1 μg/ml of incubation media produced an increase in the iron incorporation of the reticulocyte. Both LT 3 and DT 3 produced similar increases in reticulocyte iron incorporation. These results indicate that erythropoietin and triiodothyronine have different mechanisms by which erythropoiesis is stimulated. In addition, the erythropoietic stimulation produced by LT 3 and its calorigenically inactive dextroisomere was similar.

Zusammenfassung. Verabreichung von D-, wie auch von L-Triiodothyrosin erzeugt eine ähnliche Vermehrung der Erythropoiesis in nephroektomierten Ratten, wie bei der Messung der Radioeiseninkorporation durch Erythrozyten (18 h nach Injektion von radioaktivem Eisencitrat). Es ergab sich weiter, dass D- und L-Thyrosin, im Unterschied zu Erythropoieten, die reticulozytose Eisenassimilation in vitro vermehrte. Danach scheinen verschiedene Mechanismen bei der Stimulation der Erythropoiesis durch Erythropoieten und Triiodothyronin im Spiele zu sein.

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The Effect of Nickel Chloride on the Permeability of the Blood-Brain Barrier

According to the observation of EHRLICH in 1885[1] certain aniline dyes do not penetrate into the nervous system, although they stain other organs of the organism. This observation was the basis of the blood-brain barrier theory. At present, the blood-brain barrier is considered to be a multiplex mechanism regulating the exchange of certain metabolites by decreasing or enhancing transport between blood vessels and brain tissue[2-4]. Different experimental or pathological effects may cause a selective damage in some functions of the barrier[5].

Electron microscopic investigations have shown[6,7] that the cytoplasm of the endothelial cells of the capillaries in the central nervous system is continuous (not fenestrated) and surrounded by a well developed basement membrane. Between the layers of the basement membrane, pericytes and their processes are localized. Externally, glial end feet adhere to the capillaries. The electron histochemical investigations of TORACK and BARRNETT[8] indicate that there is a 5-nucleoside phosphatase activity in the glial elements and the basement membrane. In other organs and in capillaries of brain areas not protected by the barrier (choroid plexus, area postrema), nucleosid phosphatase activity can be found in the pinocytotic vesicles of the endothelial cytoplasm[9].

In this paper the question will be dealt with whether there is a correlation between the ultrastructural localizations of 5-nucleoside phosphatase in brain capillaries and the permeability of the haematoencephalic barrier. Alterations in the permeability of the blood-brain barrier were studied after specific inhibition of nucleosid phosphatase.

Our studies were performed on albino rats weighing 150–250 g. For the specific inhibition of the 5-nucleoside phosphatase, the nickel chloride inhibition technique described by KAYE[10] was used. 0.025 g/kg and 0.5 g/kg nickel chloride was administered i.p. to 25 rats. Subsequently, 5–20–60 min or 2–5–10 h, respectively, 2–5 ml of 1% trypane blue (in physiological saline) as barrier test material was injected into the tail vein of the animals. The animals were decapitated after 5–10–20 min, and 20 μ thick parasagittal sections were obtained from the vermis of the cerebellum by means of a kryostate (type Mirköz). The distribution of the dye was examined by the fluorescent method of HAMBERGER and HAMBERGER[11]. The sections were embedded in Entellan and examined with a Zeiss fluorescence microscope, using a high pressure mercury vapor lamp and BCr-12 and OG1 filters.

Following an i.v. injection of trypane blue, a wide range of capillaries can be seen under the fluorescence microscope (Figure 1). In the course of fluorescent examinations performed 30 min after the administration of 0.15 g/kg nickel chloride, it was striking that the trypane blue showing a characteristic red fluorescence leaked out of the capillaries, resulting in an intensive red perivascular fluorescence. Later the leakage of the dye increased considerabely, thus 20, 120 min after the administration, the fluorescent dye exuded from most of the capillaries and diffused profoundly into the cerebral tissue (Figures 2 and 3). However, inspite of the enzyme inhibition, the dye did not leak out of a few capillaries even at this stage. Trypane blue injected 6–10 h after administration of nickel chloride could always be detected within the vascular bed.

Our results confirm the investigations of TORACK and BARRNETT[8] according to which 5-nucleoside phosphatase plays and important role in the regulation of the permeability conditions in the capillaries of the central nervous system. According to the above authors, in the capillaries of the brain areas protected by the hematoencephalic barrier, 5-nucleoside phosphatase is not localized in the cytoplasm of the endothelial cells, but in the basement membrane and in glial elements. On the basis of the localization of the 5-nucleoside phosphatase in the basement membrane and in glial end feet, BARRNETT[12] assumes that

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