STUDY OF THE EFFECT OF HYPEROXIA ON ERYTHROPOIETIN BIOGENESIS ON AN "ENDOCRINE" KIDNEY MODEL

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An important aspect of modern experimental hematology is the question of the biogenesis of erythropoietin – the hormone which regulates erythropoiesis and which is formed chiefly in the kidneys. To elucidate the molecular mechanisms of erythropoietin formation, the writers have made use in their research of Selye's method [9] based on the use of a rat "endocrine kidney" model. An advantage of this model is the absence of excretory function in such a kidney while its internal secretory function remains intact. Accordingly, changes in biochemical processes in the "endocrine kidney" under conditions of erythropoietic stress largely reflect the mechanisms of biogenesis of erythropoietin. Data obtained previously by the use of this model to study the effect of hypoxia on the synthesis of high-polymer RNA in the kidneys and the plasma erythropoietin level showed considerable stimulation of these processes, and also their interconnection [2]. The study of these indices of the endocrine function of the kidneys under conditions of hyperoxia is interesting. It can be suggested that the depression of erythropoiesis in hyperoxia, which several authors have observed [4, 8], is the result of depression of erythropoietin synthesis in the kidney.

To shed light on this problem we investigated synthesis of high-polymer RNA in the kidneys and the plasma erythropoietin level in intact rats and also in rats with an "endocrine" kidney after exposure of the animals for 4 h in a hyperoxic chamber.

EXPERIMENTAL METHOD

Experiments were carried out on 100 noninbred male albino rats weighing 120-130 g. Synthesis of total high-polymer RNA in the kidneys and the plasma erythropoietin titer were studied in four groups of animals: 1) normal animals; 2) normal animals exposed to hyperoxia for 4 h; 3) animals with an "endocrine" kidney on the 7th day after constriction of the abdominal aorta; 4) animals with an "endocrine" kidney after exposure to hyperoxia for 4 h.

To obtain an "endocrine kidney" by Selye's method [9], the arterial blood pressure in the left kidney was reduced by partial constriction of the abdominal aorta between the origin of the two renal arteries, and as a result the kidney lost its excretory function and was transformed into an endocrine organ. The validity of the model was verified by ligation of the ureter in some of the rats, followed by determination of the presence or absence of hydronephrosis. A second control test was histological examination of the renal parenchyma.

Total high-polymer RNA was isolated from kidney tissue by the method in [10]. RNA synthesis was judged by the incorporation of adenine-8-^{14}C into RNA in the kidneys. The radioactive precursor was injected intraperitoneally in a dose of 50 μCi/100 g body weight 2 h before the animals were killed. The radioactivity of the samples was determined on a gas-flow counter.

The erythropoietin titer in the plasma was determined in 40 posthypoxic (CBA $\times$C57BL)F_1 hybrid mice on the basis of incorporation of ^{59}Fe into their erythrocytes [5].

Hyperoxic conditions were created in a gas-flow chamber with an atmosphere consisting of 95% oxygen and 5% nitrogen under ordinary atmospheric pressure and at 18-20°C.

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TABLE 1. Effect of Hyperoxia on Incorporation of Adenine-8-^{14}C (in cpm/mg RNA) into RNA of Kidneys of Intact Rats and Rats Undergoing Operation (M ± m)

Experimental condition	Kidney of intact animals	"Endocrine" kidney of animals undergoing operation	Opposite kidney
Ordinary atmosphere	30 075±1 907	18 522±1 174	23 543±1 608
Kidney of intact animals	20 323±2 073*	14 711±1 308*	17 635±1 406*

*P < 0.05.

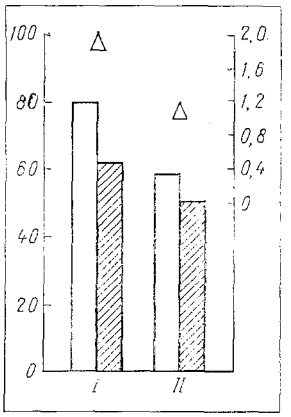

Fig. 1. Changes in plasma erythropoietin level and RNA synthesis in kidneys of rats undergoing operation under the influence of hyperoxia. I) Normal atmospheric conditions; II) hyperoxia for 4 h. Unshaded columns represent intact kidney, shaded columns "endocrine" kidney. Ordinate: left – RNA synthesis (in % of control), right – erythropoeitin level (in % of incorporation of ^{59}Fe into erythrocytes – triangles).

EXPERIMENTAL RESULTS

Investigation of synthesis of total high-polymer RNA in the kidneys of animals of groups 1 and 3 showed that RNA synthesis was reduced by 40% in the left "endocrine" kidney and by 15% in the opposite intact kidney of the rat undergoing the operation, compared with its level in normal animals. This was evidently due to the altered conditions of the hemodynamics and the absence of excretory functions in the "endocrine" kidney of the experimental rats.

After the animals had been exposed for 4 h in the hyperoxic chamber, synthesis of high-polymer RNA in the kidneys of the intact rats (group 2) was reduced by 34% compared with the control (Table 1). The plasma erythropoietin titer of these animals was at the same level as in the rats of group 1 (the percentage incorporation of ^{59}Fe was 1.03). In the animals undergoing the operation, after exposure to hyperoxia (group 4) a decrease was observed in the rate of incorporation of the radioactive precursor into RNA of the "endocrine" kidney by 21%, compared with a decrease of 24% in the opposite (right) kidney (Fig. 1).

A raised erythropoietin level was found in the plasma of the animals on the 7th day after the operation (incorporation of ^{59}Fe into the erythrocytes of the posthypoxic mice was 1.92%), as many workers have observed during changes in the hemodynamics in the kidneys [1, 6]. However, exposure of the rats of this group to hyperoxic conditions for 4 h led to a fall in their plasma erythropoietin titer by 50% (Fig. 1). It can thus be concluded that inhibition of erythropoiesis during hyperoxia takes place primarily on account of a decrease in the plasma erythropoietin concentration, and it is only the limited sensitivity of the method used which pre-

vented determination of the decrease in the erythropoietin titer in the intact rats. However, the depression of synthesis of high-polymer RNA observed during hyperoxia in both the "endocrine" and the intact kidney indicates that a short period of hyperoxia is sufficient to change the chain of biosynthetic reactions at the genome level leading to a reduction in formation of the hormone or of its precursor [7]. Fractionation of RNA in the kidneys, carried out in the writers' previous experiments [3], showed that hyperoxia for 4 h caused the maximal decrease in synthesis of DNA-like RNA [3]. The connection between processes of RNA synthesis in the kidney and its endocrine function, confirmed also by the "endocrine kidney" model, is evidence that DNA-dependent RNA synthesis is one of the mechanisms included in erythropoietin biogenesis.

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BIOCHEMICAL AND MORPHOLOGICAL CHANGES IN LUNG AND LIVER TISSUES OF ALBINO RATS WITH EXPERIMENTAL PARAQUAT POISONING

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Metabolic conversions of the herbicide paraquat (1,1-dimethyl-4,4-bipyridyl dichloride) are carried out with the participation of a microsomal NADPH-dependent system of oxidases of mixed function [3]. It has been suggested [4, 5] that during conversion of paraquat in vivo superoxide radicals responsible for the specific toxic action of the compound are formed.

The object of this investigation was to study the effect of paraquat on the ability to form free radicals in the tissues of the lungs and liver and on superoxide dismutase (SOD) activity, an enzyme with whose participation superoxide radicals are detoxicated, and to study pathomorphological changes in these organs, developing under the experimental conditions used.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-200 g. The animals were poisoned by peroral administration of paraquat in three doses, each of 25 mg/kg, or in repeated doses of 12.7 and 6.35 mg/kg daily for 30 days, equivalent to 0.2, 0.1, and 0.05 LD_{50}. The animals were decapitated 1, 5, and 30 days

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