Disturbances of Hypoxanthine Metabolism in the Liver of Resuscitated Rats

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Catabolism of purine mononucleotides to hypoxanthine and xanthine in the liver is enhanced in rats resuscitated after a 6.5-min asphyxia. Reduced incorporation of ^{14}C-hypoxanthine into these nucleotides 30 min, 1, and 3 days after resuscitation attests to its disturbed reutilization. This probably promotes activation of the xanthine oxidase reaction, leading to purine deficit and hyperproduction of reactive oxygen species.

Key Words: *hypoxanthine; postresuscitation disturbances*

Accumulation of hypoxanthine (HX) in tissues during the postresuscitation period results from enhanced catabolism of purine mononucleotides [3,4]. Activation of xanthine oxidase reaction renders this process irreversible [9]. Moreover, it has been previously hypothesized that *in vivo* activation of xanthine oxidase reaction leads to hyperproduction of reactive oxygen species [3,4,6]. An alternative pathway is reutilization of HX. Apart from hepatogenic HX, the liver metabolizes HX delivered by the blood [14], therefore disturbances in HX metabolism can have grave consequence for the whole organism. In the present study we evaluated the efficiency of HX reutilization in the liver of resuscitated rats.

MATERIALS AND METHODS

Experiments were carried out on 140 male rats weighing about 200 g. They were resuscitated after 6.5-min asphyxia as described previously [8]. They were narcotized with ether 30 and 90 min, 6 and 24 h, and 3, 7, and 21 days after resuscitation, a and the liver was *ex vivo* fixed in liquid nitrogen. Control rats were subjected to the same procedures except for asphyxia and resuscitation. ^{14}C-HX was injected intravenously in a dose of 740 kBq/kg body weight 25 min prior to fixation. The liver was processed as described elsewhere [5].

It is known that HX is converted not into inosine, but into inosine monophosphate and then into other nucleoside-monophosphates (NMP): adenylosuccinate, adenosine monophosphate, xanthosine monophosphate, guanosine monophosphate [9]. NMP are then phosphorylated to nucleoside di- and triphosphates (NDTP). Hence, reutilization ^{14}C-HX can be assessed only by measuring radioactivity not only of major mononucleotides (ATP and GTP) but also of all purine mononucleotides; NDTP and NMP should be measured as two independent pools. The content of HX and xanthine in the liver was measured as described elsewhere [13], and label incorporation into purine NMP and NDTP and their content were determined by our methods. The data were processed statistically using the Student and Wilcoxon—Mann—Whitney tests and Spearman correlation coefficient [2].

RESULTS

Catabolism of NMP and NDTP in the liver of resuscitated rats is enhanced, as evidenced by reduced content of these nucleotides and accumulation of HX and xanthine (Table 1). Hypoxanthine is a common catabolite of all purine mononucleotides [11,12]. It can be hypothesized that HX formed during asphyxia

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TABLE 1. Parameters of Purine Metabolism in the Liver of Rats Resuscitated after Mechanical Asphyxia ($M \pm m$)

Group (n=7-18)	Content in the liver			Incorporation of ^{14}C-hypoxanthine		
	NDTP	NMP	hypoxanthine+ xanthine, nmol/g protein	hydrochloride extract of the liver	NDTP	NMP
	optical density units/g liver			counts/sec/g liver		
Control	88.7±5.8	19.9±1.0	470±40	3158±225	510±73	241±26
30 min	68.1±4.5**	17.2±1.1*	827±40****	2750±212	153±33****	144±34*
90 min	59.4±13.9*	18.1±2.0	642±39**	5181±398****	580±189	400±74*
6 h	68.8±11.8	16.6±2.4	500±12	3372±635	443±231	160±38
24 h	74.4±16.8	19.6±2.5	592±17**	3339±362	166±41****	161±24*
3 days	79.3±7.8	19.7±2.1	598±25**	4793±566***	180±65***	185±40
7 days	75.1±15.5	18.0±3.4	872±55*	3343±368	384±158	230±61
21 days	78.1±11.3	21.2±2.2	977±28**	3630±386	484±160	236±50

Note. *p<0.05, **p<0.01, ***p<0.005, ****p<0.001 compared with the control.

is delivered to the liver and reutilized after restoration of circulation. This could compensate for the purine loss. However, during the first 30 min after resuscitation the total radioactivity of hydrochloride extract from the liver did not differ from the control (Table 1). This is probably due to the fact that cell HX uptake depends on its intracellular reutilization [9], which is very limited during this period as evidenced by low ^{14}C-HX incorporation into NMP and NDTP. Ninety minutes after resuscitation, ^{14}C-HX incorporation surpassed the control level by 66%, in parallel, total radioactivity increased by 64%. There is a strong correlation between these parameters (r_s=0.9). Correlation analysis within each group is a more adequate statistical approach in such experiments then comparison between control and experimental groups, because of their heterogeneity caused by death of some animals (ineffective resuscitation constituted 36%, postresuscitation mortality varied from 19 to 71% on the 30th min and on the 21st day, respectively).

The hypoxanthine phosphoribosyl transferase reaction is a limiting stage of HX conversion to NMP [9,14]; however, the exact limiting factors have not been identified. M.-F. Vincent *et al.* allocate this role to NMP and NDTP. Being added in physiological concentrations to an *in vitro* system, these substances inhibit liver hypoxanthine phosphoribosyl transferase by 95% [14]. However, the decrease in the content of NMP and NDTP in our experiments was accompanied by inhibition, but not activation of ^{14}C-HX reutilization. The absence of negative correlation between ^{14}C-HX incorporation into NMP and the content of NMP and NDTP in all experimental groups suggests the existence of an unknown *in vivo* limiting factor such as insufficient biosynthesis of phosphoribosyl diphosphate or its 6-7-fold enhanced utilization in *de novo* purine synthesis soon after a 6.5-min asphyxia [5].

Inhibition of HX reutilization and accumulation of HX and xanthine, substrates of xanthine oxidase are prerequisites of its activation. Despite the fact that activity of dehydrogenase form of xanthine oxidase *in vitro* surpassed that of oxidase form [1,7, 10,14], the *in vivo* conditions are more favorable for the oxidase form. Even total ischemia cannot reduce tissue oxygen content to the level when the oxidase reaction is inhibited [1,9], whereas dehydrogenase reaction is readily inhibited with the rise of NADH/NAD ratio [10,14] typical of ischemia and postischemic period [1,14]. Under these conditions, oxidase mechanism of HX oxidation dominates over the dehydrogenase one.

Thus, the development of the postresuscitation energy deficiency is related not only to enhanced catabolism of purine mononucleotides, but also to impaired reutilization of HX. This results in activation of xanthine oxidase reaction and leads to irreversible purine loss and hyperproduction of reactive oxygen species.

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Free Radical Oxidation and Antioxidant Activity in Placental Tissue in Preterm Labor

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Effect of specific scavengers of reactive oxygen species on free-radical oxidation is studied in central and peripheral zones of the placenta from preterm delivering women (32-36 weeks) using the chemiluminescence method. A lower contribution of hypochlorite into free-radical processes and a reduced content of nonprotein SH-groups in the placenta are observed, superoxide dismutase, catalase, and total antioxidant activity being unchanged. It can be assumed that the reduced contribution of hypochlorite into free-radical processes is partially responsible for impaired antimicrobial barrier between mother and fetus in preterm labor.

Key Words: *free-radical oxidation; chemiluminescence; antioxidant activity; placenta; preterm labor*

Placental insufficiency is largely responsible for premature labor and fetal abnormalities. An important role in the pathogenesis of premature labor is played by free-radical oxidation (FRO) in the placenta, impairing the structure and permeability of cell membranes [1,5]. These destructive processes result from the imbalance between the intensity of FRO and efficiency of the antioxidant defense system and can be caused by a number of factors, in particular, endocrine insufficiency and fetal hypoxia due to impaired oxygen supply.

Mechanisms of generation of reactive oxygen species and activity of the antioxidant defense system of different placental zones in women with normal and abnormal (premature labor) pregnancy remain poorly understood.

In light of this, chemiluminescence technique in combination with other methodical approaches allows one to evaluate the role of free radicals in FRO and activity of antioxidant defense system in placental tissue in women with normal and abnormal (preterm labor) pregnancy.

MATERIALS AND METHODS

Placentae were obtained from women with normal pregnancy and term labor and from women with

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