Mechanism of Mildronate Effect on Rabbit Fetuses Developing under Conditions of Placental Insufficiency

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Mildronate, 3-(2,2,2-trimethyl hydrasinium)propionate dehydrate, a drug producing antihypoxic and selective vasodilating (in vascular spasm) effects, improves the status of rabbit fetuses with chronic intrauterine hypoxia induced by experimental placental insufficiency. The drug has an unfavorable effect on fetuses developing under conditions of normal uteroplacental circulation. The results are based on physiological and biochemical investigations of fetuses and placentas.

Key Words: *hypoxia; rabbit fetuses; placental insufficiency; mildronate*

Disorders of the central nervous system caused by episodes of intrauterine hypoxia cause perinatal morbidity and mortality. Antihypoxants modulating the intensity of oxidative metabolism have been widely used for treating fetal hypoxia.

Carnitine-dependent system of fatty acid activation and transport plays a key role in the regulation of exogenous oxygen consumption. Disorders of oxygen delivery to cells lead to accumulation of active forms of nonoxidized fatty acids, the derivatives of acyl carnitine and acyl coenzyme A. Carnitine activates fatty acids by forming their transport forms, acyl carnitines. Normally, the levels of acyl carnitine production and oxygen consumption in cells are balanced; in oxygen insufficiency, the inflow of fatty acids should be decreased [2]. So, the drugs reversibly limiting carnitine biosynthesis hold good promise in the treatment of chronic fetal hypoxia.

Mildronate, 3-(2,2,2-trimethyl hydrasinium) propionate dehydrate produces antihypoxic, cardioprotective, thrombolytic, antisclerotic, and immunomodulating effects and stimulates the central nervous system [2,5]. Mildronate has a selective vasodilating effect only on constricted vessels without inducing the "stealing syndrome".

We studied the effect of mildronate on the development of rabbit fetuses with chronic intrauterine hypoxia and some mechanisms of the drug effect under these conditions.

MATERIALS AND METHODS

The study was carried out on 14 female Chinchilla rabbits weighing 3-5 kg and 83 fetuses. Placental insufficiency was induced on day 18 of gestation by ligating 1/3 preplacental uterine vascular branches near each fetal sack of one uterine horn. The fetuses in the other uterine horn were left intact. Intravenous injections of 10% mildronate into the lateral ear vein were started immediately after the operation and were continued for 10 subsequent days. On day 29 of gestation, the animals were sacrificed. Live and dead fetuses were counted; fetuses and placentas were weighed. The following parameters

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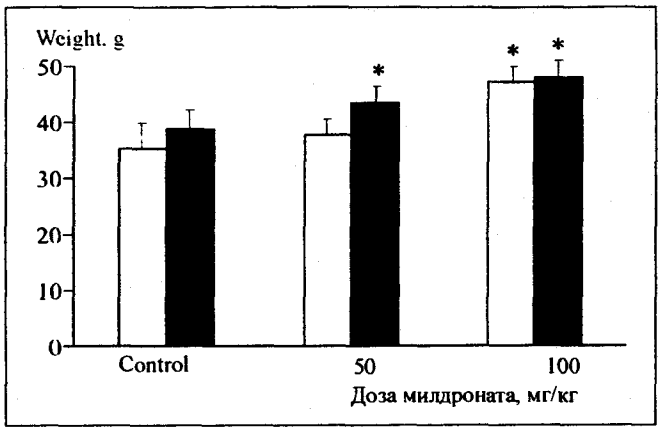

Fig. 1. Mean weight of fetuses in experimental (dark bars) and intact (light bars) uterine horns after injection of 0.9% NaCl (control) and 10% mildronate. Here and in Figs. 2 and 3: *$p<0.05$ *vs.* the control.

of placentas and fetal brains were studied: luminol-dependent chemiluminescence (LCL) [7] reflecting the intensity of production of active oxygen forms, total antioxidative activity (AOA) by the chemiluminescent method with riboflavin as the source of fluorescence, activities of glutathione peroxidase [3] and catalase [6], and the L-arginine-inhibited component of NADPH-diaphorase activity, an indirect indicator of the activity of nitric oxide synthetase (NOS) [4,8].

In the first series of experiments, mildronate was injected into the females in a dose of 50 mg/kg, in the second in a dose of 100 mg/kg. In the third (control) series the females were injected with 0.9% NaCl in a dose of 1 ml/kg.

The results were statistically processed using the standard statistical analysis methods: descriptive statistics and unifactorial dispersion and multiple regression analyses. The groups were compared using Student's *t* test, nonparametrical Wilcoxon's *U* test, and Pearson's and Spearman's correlation coefficients. The mean weight of a fetus was calculated from the female body weight, weight of fetuses in the contralateral horn, and weight of placentas of examined fetuses. Allowances were made using covariation analysis.

RESULTS

The weight of fetuses in experimental uterine horns increased with a higher mildronate dose, while in intact horns it increased only after 100 mg/kg mildronate (Fig. 1). The mean weight of placentas did not change.

The percentage of dead fetuses in experimental and intact uterine horns was virtually the same after injection of normal saline (22.2 and 16.7%, respectively). The mortality of experimental and intact fetuses after mildronate was different. In uterine horns with placental insufficiency, mortality decreased, though negligibly: by 10% after 50 mg/kg and by 9.1% after 100 mg/kg mildronate. In intact horns mortality increased with increase in mildronate dose: 30.4 and 56.6%, respectively, $p<0.01$.

Analysis of biochemical parameters in the third series of experiments showed moderate hypoxia in fetuses developing under conditions of induced placental insufficiency. A tendency to an LCL increase was observed in the placentas of these fetuses in comparison with intact ones, which indicated increased generation of active oxygen forms and lack of correlation between AOA and glutathione peroxidase in intact placentas. NOS activity was 1.8-fold higher in experimental placentas ($p<0.01$, Fig. 2), stimulating the production of NO, a vasorelaxation factor which can be regarded as a component of the compensatory reaction aimed at the maintenance of an adequate blood flow in the placenta and inactivation of excessive active oxygen forms by reacting with superoxide anion radicals. The brain of such fetuses was more resistant to hypoxia than the placenta: NOS activity was not changed in the brain, which confirms out previous data [10]. Only the catalase level was decreased in the brain of intact fetuses in comparison with experimental fetuses ($p<0.05$).

The effects of mildronate on the biochemistry of the placenta and brain of experimental and intact fetuses were different. LCL was decreased in the placentas exposed to chronic hypoxia: by almost 1.4-fold after 50 mg/kg (a tendency) and 2-fold after 100 mg/kg ($p<0.01$) in comparison with control placentas. The activities of the antioxidative enzymes glutathione peroxidase and catalase decreased. Catalase activity in the placenta after both doses of mildronate decreased by almost 1.5 times and in the brain by 1.3 times in comparison with the control (Fig. 3). Similar changes were observed in the brain glutathione peroxidase levels. In the placenta, the level of glutathione peroxidase showed a tendency to a decrease only after a mildronate dose of 50 mg/kg. These shifts are confirmed by strong correlation between activities of glutathione peroxidase and catalase (in the placenta in all experimental groups: $r=0.835$-0.903 and in fetal brain after a dose of 100 mg/kg, $r=0.932$). Such a decrease in the activities of both enzymes may indicate a decreased production of H_2O_2 under the effect of the drug, because their activity is determined by hydrogen peroxide degradation. Changes in the activity of glutathione peroxidase may be related to decreased level of reduced glutathione, the main factor limiting its activity.

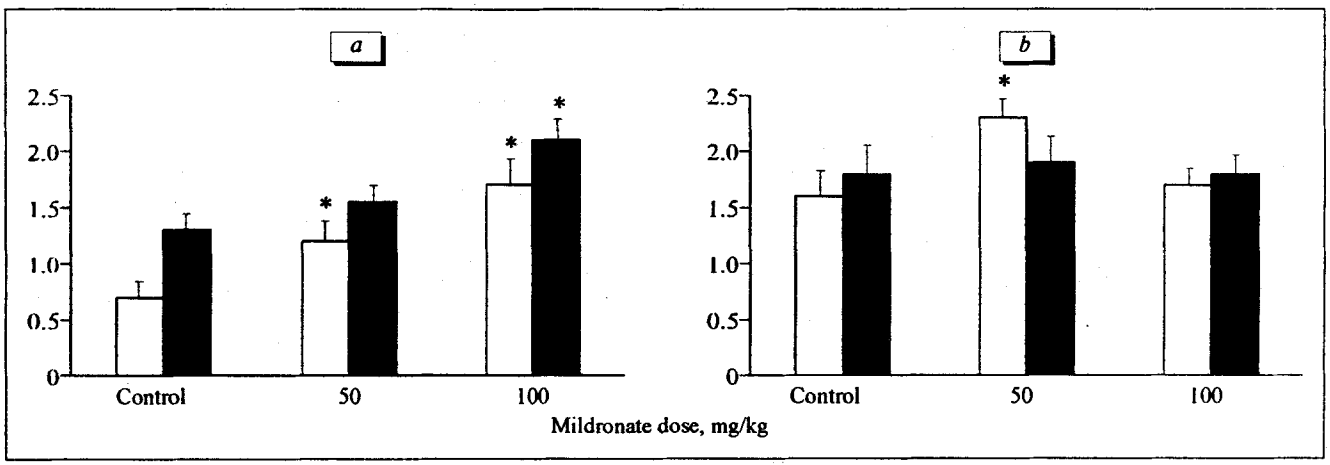

Fig. 2. Nitric oxide synthetase activity in the placenta (*a*) and brain (*b*) of experimental (dark bars) and intact (light bars) fetuses. Ordinate: nmole nitroblue tetrasolium-formazane/(mg protein×min).

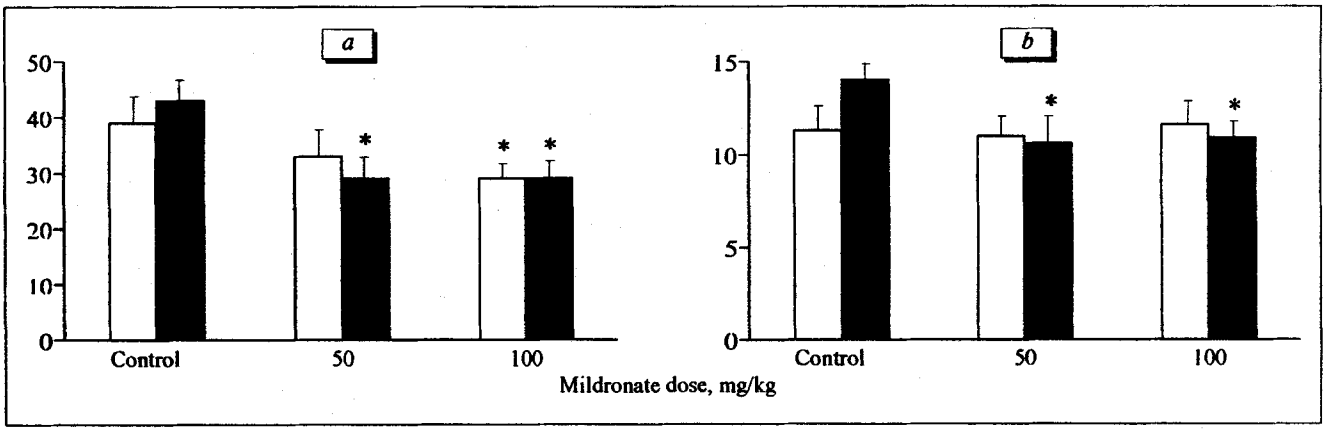

Fig. 3. Catalase activity in the placenta (*a*) and brain (*b*) of experimental (dark bars) and intact (light bars) uterine horns. Ordinate: mmole H_2O_2/(min×mg protein).

In experimental fetuses' placentas, a significant decrease in NOS activity (by 1.67 times) was observed only after a high dose of mildronate that created favorable conditions for vasorelaxation and blood supply to the placenta after ligation of placental vessels. NOS activity in the brain of these fetuses did not change.

Mildronate changed the studied biochemical parameters of fetuses developing under conditions of intact circulation. Catalase activity in intact placentas slightly decreased (by 1.3 times) only after 50 mg/kg mildronate. Glutathione peroxidase activity in the placentas was similar to that in experimental fetuses ($p<0.05$). Activities of catalase and glutathione peroxidase in the brain of these fetuses did not change. NOS activity in the placenta increased in a dose-dependent manner: by 1.74 times after a lower dose and by 2.46 times after a higher dose. In the brain, NOS activity increased after 50 mg/kg mildronate ($p<0.01$, Fig. 2). Under conditions of normoxia and a stable level of active oxygen forms the activity of NOS increased, leading to production of excessive nitric oxide, which may damage the tissues [11].

Our results indicate different effects of mildronate on the fetus under conditions of impaired and normal uteroplacental circulation. The drug effect is observed only in fetuses developing under conditions of impaired uteroplacental circulation, which is confirmed by increment in their weight and changes in biochemical parameters: decreased LCL and increased NOS activity in the placenta (after a dose of 100 mg/kg) and decreased activities of catalase and glutathione peroxidase in the placenta and brain of fetuses. The study revealed changes indicative of unfavorable effect of the drug on the fetus developing under conditions of normal uteroplacental circulation (increased mortality and a dose-dependent excessive increase in NOS activity in the placenta and even in fetal brain). Our study confirmed the assumption that drugs affecting the uteroplacental circulation are indicated only in cases of impaired blood flow in the mother-placenta-fetus system. According to published reports,

drugs decreasing the uteroplacental blood flow in normal pregnancy impair the development of the fetus [1,9].

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