LITERATURE CITED

1. I. A. Drzhevetskaya, Endocrine System of the Growing Organism [in Russian], Moscow (1987).
2. V. P. Kulik, V. K. Novikov, T. P. Pisareva, et al., Sov. Med., No. 7, 16 (1987).
3. Diabetes Mellitus. WHO Expert Report [Russian translation], Moscow (1987), pp. 22-26.
4. E. P. Shuvalova (ed.), Tropical Diseases [in Russian], Moscow (1989), p. 460.
5. N. A. Tyurin et al., Diseases of the Newborn and Infants [in Russian], Moscow (1986), p. 79.

EFFECT OF CORTICOSTEROID IMBALANCE ON THE CATECHOLAMINE SYSTEM OF THE FETAL RAT BRAIN

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Administration of glucocorticoids which pass through the placenta is used in medical practice during pregnancy to prevent the syndrome of respiratory disturbances in the newborn [4]. Meanwhile, in children exposed during early development to a high glucocorticoid level as a result of hormone therapy of the mother or to the action of stressors, disturbances of neuropsychological and behavioral development are observed [7]. In model experiments on animals, psychoemotional disturbances also are found in the offspring, due evidently to changes in function of the catecholamine mediator system of the brain [2]. However, it is not clear what changes take place in the mediator system of the fetal brain when the hormone balance is disturbed. Solving this problem would help to establish the role of glucocorticoids in prenatal development of the catecholamine mediator system and explain the mechanism of realization of the remote aftereffects of damaging influences of the environment and of hormone therapy in early ontogeny on the functions of the adult.

The aim of this investigation was to study levels of catecholamines and activity of the key enzyme of their biosynthesis, namely tyrosine hydroxylase (TH) in the fetal rat brain after disturbance of the glucocorticoid balance in the blood of the pregnant mothers.

EXPERIMENTAL METHOD

Female Wistar rats received a subcutaneous injection of corticosterone ("Calbiochem," 2.5 mg/0.2 ml/100 g), the corticosteroid synthesis blocker metyrapone (Metypirone, CIBA, 12 mg/0.2 ml/100 g), or distilled water (0.2 ml/100 g), subcutaneously on the 16th and 18th days of pregnancy, and the 21-day fetuses of these groups of females were studied 3 days later. In other experiments, on the 20th day of pregnancy the mothers were given corticosterone in the same dose or were left intact, and their fetuses were studied 6 h later. After sacrifice of the mothers, the fetuses were removed by cesarean section and quickly weighed. The brain was isolated in the cold and cut along a plane passing from the pineal gland to the optic chiasma into brain stem and anterior parts. Some of the brain tissue samples were used for fluorometric measurement of the DNA concentration [12] and of protein, by Lowry's method [13]. The dopamine and noradrenalin concentrations or TH activity [1] were determined fluorometrically in other samples, in the presence of saturating concentrations of coenzyme ($DMPH_4$) and tyrosine.

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TABLE 1. Body Weight, Weight of Brain, Protein, DNA, and Catecholamine Content, and TH Activity in Brain of 21-Day Rat Fetuses after Injection of Substances Altering the Blood Corticosteroid Levels into Females on 16th and 18th Days of Pregnancy (M ± m)

Parameter studied	Substances injected into females		
	water	corticosterone	metyrapone
Body weight, g	3,69±0,04(40)	3,07±0,05(34)*	3,28±0,05(47)*,**
Weight of brain, mg:			
brain stem	77,7±4,47(10)	73,9±2,85(21)	73,6±2,54(20)
anterior part	58,1±4,69(13)	62,5±2,52(24)	57,1±2,52(18)
Protein concentration in brain, mg/g:			
brain stem	77,3±7,6(7)	76,4±4,8(9)	70,9±3,8(10)
anterior part	82,2±6,9(6)	78,7±4,6(11)	75,8±8,3(10)
DNA concentration in brain, mg/g:			
brain stem	4,08±0,17(7)	4,45±0,22(9)	4,27±0,26(10)
anterior part	5,29±0,48(6)	5,30±0,24(11)	5,35±0,39(10)
TH activity in brain, pmoles dopa/min/mg protein:			
brain stem	9,63±0,94(10)	6,94±0,34(16)*	5,72±0,39(15)*,**
anterior part	4,39±0,43(10)	3,88±0,22(15)	3,68±0,22(15)
Dopamine concentration in brain, μg/g:			
brain stem	0,28±0,05(3)	0,29±0,03(11)	0,25±0,03(11)
anterior part	0,27±0,06(6)	0,40±0,06(13)	0,31±0,03(9)
Nonradrenalin concentration in brain, μg/g:			
brain stem	0,24±0,06(3)	0,24±0,02(13)	0,21±0,01(12)
anterior part	0,20±0,02(7)	0,25±0,03(13)	0,21±0,02(9)

Legend. Number of observations in parentheses; *$p < 0.05$ compared with injection of water; *$p < 0.05$ compared with injection of corticosterone.

EXPERIMENTAL RESULTS

Disturbance of the blood glucocorticoid levels in pregnant rats, whether in upward or downward directions, inhibited the general development of the rat fetuses, judging by their body weight (Table 1). These results agree with those obtained by other workers who found a decrease in body weight of fetal and neonatal animals developing under conditions of corticosteroid excess or deficiency, while their pregnant mothers received hormones or underwent adrenalectomy [10]. Corticosteroids inhibit biosynthesis of several proteins, but at the same time they induce production of certain specific enzymes [6]. It is evident, therefore, that both a decrease and an increase in corticosteroid concentrations can block synthesis of certain proteins and thereby inhibit the general development of the animal. Meanwhile disturbance of the corticosteroid balance had no marked teratogenic effect on the brain. The weight of the brain and its protein and DNA concentrations, characterizing metabolic and proliferative processes to some extent [3], were about equal in the 21-day fetuses in all the experimental groups (Table 1).

TH activity in the brain stem of the 21-day-old fetuses was reduced (Table 1), as also was their body weight, as a result of a rise or fall of the blood corticosteroid level of the female rats on the 16th and 18th days of pregnancy. These facts indicate that lowered TH activity may be one manifestation of delay in development of the organism as a whole. Meanwhile corticosterone caused more marked inhibition of general development of the fetuses than metyrapone. However, TH activity in the brain stem of fetuses developing with exposure to an excess of corticosteroids was much greater than in fetuses deficient in these hormones. TH activity of the fetal brain after disturbance of the maternal hormonal balance is evidently an integral reflection of a change in the rates of general development of the animal and the specific effect of corticosteroids on the brain catecholamine system. Higher TH activity in the fetuses in response to injection of corticosterone compared with metyrapone suggests that besides the inhibitory effect there is also a stimulating effect of glucocorticoids on the enzyme, which is manifested to some degree even when general development of the animal is inhibited.

The dopamine and noradrenalin levels in the brain and TH activity in the anterior part of the brain (Table 1), where terminals of catecholamine neurons are located, were unchanged in the rat fetuses when the corticosteroid balance was disturbed. The tissue level of the mediator is determined not only by its biosynthesis, but also by its accumulation and destruction. The mechanisms responsible for these processes have natural rules governing their development and definite ability for mutual compensation [5]. The absence of any change in TH activity in terminals of the catecholamine neurons of

TABLE 2. Body Weight and Brain TH Activity of 20-Day Rat Fetuses 6 h after Injection of Corticosterone into Mothers (M ± m)

Group of fetuses	Body weight, g	TH activity in brain, pmoles dopa/min/mg protein	
		brain stem	anterior part
Control	2,49±0,04(39)	5,58±0,41(32)	3,33±0,32(31)
Hormone	2,51±0,03(61)	6,74±0,34(51)*	3,54±0,27(46)

Legend. Number of observations in parentheses; *$p < 0.05$ compared with control.

the rat fetal brain when the blood hormone levels of the mother are disturbed may be due, in particular, to the later times of their postnatal formation compared with the perikaryon.

TH activity in the brain stem of 20-day-old rat fetuses was increased 6 h after injection of corticosterone into their mothers, when the inhibitory action of the hormone on general fetal development, judging by their body weight, has not yet begun to appear (Table 2). The stimulating effect of corticosteroids on brain TH activity also is observed when glucocorticoids are injected into animals soon after birth [8, 15]. Incidentally, in the early postnatal period, just as during intrauterine development, in the present experiments corticosteroids activated TH in the perikaryon, but had no appreciable effect on terminals of brain catecholamine neurons [15]. Data obtained by these workers and the results of our own experiments show that the end of prenatal and the beginning of postnatal ontogeny are periods of development of the brain catecholamine system that are sensitive to disturbance of the glucocorticoid balance. It will therefore be evident that injection of glucocorticoids into pregnant [2] or newborn animals [14] will have a prolonged effect on the catecholaminergic system of the brain in adult animals.

Thus activity of TH, the key enzyme of catecholamine biosynthesis, in the region of the perikaryon in rat fetal brain neurons depends on the maternal blood corticosteroid level during pregnancy, and this may be one cause of the psychoemotional disturbances of the adult offspring. Inhibition of activity of the enzyme after disturbance of the hormonal balance may be connected with delayed development of the animal as a whole. Meanwhile, corticosteroids have evidently a stimulating action directly on TH during the period of intrauterine development.

LITERATURE CITED

1. N. N. Dygalo, Vopr. Med. Khim., **33**, No. 4, 126 (1987).
2. N. N. Dygalo and E. V. Naumenko, Ontogenez, **19**, No. 3, 319 (1988).
3. E. V. Maksimova, Neuroontogeny [in Russian], Moscow (1985), p. 6.
4. N. I. Puzyreva, R. M. Laryushkina, and N. K. Ryzhkova, The Respiratory Distress Syndrome and Lung Surfactant in the Newborn [in Russian], Moscow (1987).
5. V. V. Raevskii, Neuroontogeny [in Russian], Moscow (1985), p. 199.
6. V. B. Rozen, Principles of Endocrinology [in Russian], Moscow (1984).
7. O. Benesova, J. Petova, and A. Pavlik, Brain Maldevelopment and Drugs, Prague (1984).
8. J. A. Diez, P. Y. Sze, and B. E. Ginsburg, Neurochem. Res., **2**, 161 (1977).
9. J.-P. Dupouy, Neuroendocrinology, **16**, 148 (1974).
10. J.-P. Dupouy, H. Coffigny, and S. Magre, J. Endocr., **65**, 347 (1975).
11. M. Jacobowitz and J. S. Richardson, Pharmacol. Biochem. Behav., **8**, 515 (1978).
12. C. Labarca and K. Paigen, Analyt. Biochem., **102**, 344 (1980).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., **193**, 265 (1951).
14. C. Nyakas, J. Viltsek, and E. Endroszi, Catecholamines and Stress: Recent Advances, Amsterdam (1980), p. 375.
15. P. Sze, K. Markey, and A. Towle, Endocrinology, **111**, 1519 (1982).