ОГЛЯДИ

The developmental neuroendocrinology of reproduction and adaptation: lessons from animal research

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In order to commemorate the 30th anniversary of the establishing Department of Endocrinology of Reproduction and Adaptation at the V.P. Komisarenko Institute of Endocrinology and Metabolism (Kyiv, Ukraine), the results of animal research in the field of developmental neuroendocrinology of reproduction and adaptation in early ontogenesis are reviewed in the article. Special attitude is paid to sex differentiation of the brain and developmental programming of hypothalamic-pituitary-adrenal axis. Presented are reprogramming effects of perinatal steroids, stress, some drugs, and chemical endocrine disruptors on the developing brain. Phenomenology and neurochemical mechanisms underlying hormone-neurotransmitter imprinting of morphology of the hypothalamus, sexual behavior, reproductive and endocrine functions, and stress reactivity are under discussion. The results of the studies could contribute to prenatal prevention of neuroendocrine and behavioral disorders.

Key words: brain; sexual differentiation; androgens; estrogens; glucocorticoids; neurotransmitters; prenatal stress; sexual behavior; stress reactivity; endocrine disruptors; rat.

INTRODUCTION

This article has been written to commemorate the 30th anniversary of the establishment of the Department of Endocrinology of Reproduction and Adaptation and to present its experience in the field of developmental neuroendocrinology. Its predecessor was the Laboratory for Neurohormonal Regulation of Reproduction, organized in 1973. During those decades, investigation of etiology and pathogenesis of the early and long-term neuroendocrine and behavioral effects of perinatal influences on developing brain remained in the focus of our research team (Photo). This article reviews the main results of these studies.

In general, there are two main pathways for the participation of hormones, neurotransmitters, cytokines, metabolites, calcium ions, and other factors in brain programming in early life. The first is their genome-determined direct impact on neurogenesis – the formation of nerve and neuroendocrine structures during embryonic morphogenesis. It is realized by proliferation and apoptosis of neuroblasts and neurocytes, their migration, myelination of nerve fibers, synaptogenesis, growth of dendrites and axons. The second way is epigenetic mechanisms, which can modify developmental genes expression and translation processes [1-3]. As well, epigenetic agents can remodel chromatin by altering nucleosome density.

Programming of physiological functions in early ontogenesis is the basic biological law of the development of human beings and other mammals. Particularly, it involves neuroendocrine and behavioral systems [4-7]. They are open to injury in the case of early-life pathological conditions. Both these processes are underlying with hormone-neurotransmitter imprinting of the developing brain due to epigenomic modifications during specific critical periods. Imprinting mechanisms can be traced to the effects of prenatal stress, hormonal imbalance, certain drugs, endocrine disruptors, alimentary

factors, etc. Their consequences are an alteration of the density of tissue distribution and binding ability of the receptors of steroids and other hormones, synthesis, metabolism, and signaling of neurotransmitters and neuropeptides – noradrenaline, serotonin, dopamine, acetylcholine, opioids, vasopressin, so on. There are changes in the expression of protein synthesis and phosphorylation.

The concept of hormone-neurotransmitter imprinting of developing brain was the starting point of our research. The researchers explore two most widely spreading animal models for studying physiology and pathology of sexual differentiation of the brain (SDB): prenatal stress (PS) syndrome in male rats and neonatal androgenization of female rats. The first of these is characterized by demasculinization/feminization of neuroendocrine control of reproduction and sexual behavior, and modification of the hypothalamus-pituitary-adrenal (HPA) axis function. In the second case, defeminizing/masculinizing of developing brain is going on. In both cases, the basis of the disorders is the deviation in androgen-dependent SDB.

Androgen-dependent sex brain differentiation

In both sexes, an immature brain is potentially capable of developing along with the female type. In adulthood, its manifestations are female sexual behavior and the ability for pre-ovulation estrogen-induced surge of pituitary LH. Fetal testicular (in males) or exogenous (in females) androgens program hypothalamic and other neuroendocrine centers of sexual behavior and regulation of gonadotropin secretion towards male type. In rodents (rats, mice, hamsters), the critical period of SDB covers the last days before birth and a few postnatal days (PND). Early research by our team aimed at clarification of pathogenesis of early life androgen-induced endocrine and behavioral abnormalities in female rats [8-12].

Newborn Wistar female pups were given testosterone propionate (TP) oil solution subcutaneously in daily doses of 150 mcg to 1.25 mg per animal once or repeatedly. Subsequently, they developed permanent estrus or a dramatic violation of the estrous cycles (persistent or irregular estrus), as well as polycystic ovaries. The contents of progesterone

Photo. From left to right: P.V. Sinitsin, L.I. Polyakova, N.D. Nosenko, A.G. Reznikov, L.V. Chaykovskaya, L.V.Tarasenko

and 20α-hydroxyprogesterone in the ovaries, isolated with two-dimensional thin-layer chromatography in Silicagel, reduced significantly. Blood plasma progesterone levels measured by radioimmunoassay in females with persistent estrus decreased significantly as well. With moderate androgenization, estradiol-17β levels decreased compared with those in normal animals and were increased in animals after the highest dose of TP. Surprisingly, LH levels in plasma that have been determined with the rat LH radioimmunoassay did not change, while contents of bioactive LH in the adenohypophysis and LH-RH in the hypothalamus decreased. In neonatally androgen-sterilized females, which were ovariectomized at age of 4-5 months, the hypothalamus, adenohypophysis, and uterus tissue binding of 6,7-3H-estradiol-17β with high specific activity (10 Ki/mmole) were significantly reduced.

In parallel with disorders of neuroendocrine control of reproductive functions, in neonatally androgenized females (NAF), the programming of HPA axis function is modified. It was suggested that the sex differences of HPA axis function in adults might be the result of sex hormone-dependent organization of the brain [13]. In rats, there is a coincidence in time between the organizing effect of sex hormones on the brain and the maturation of HPA axis [14]. Investigation of the mechanisms that cause long-term disorders of HPA axis in NAF has shown that they are accompanied by changes in the expression of corticoliberin mRNA and the content of this neurohormone in the hypothalamus, as well as an increase in the content of glucocorticoid receptor in paraventricular nuclei [15].

We studied the condition of HPA axis in 6-month-old female rats with persistent estrus who received 250 mcg TP on PND 3. Blood plasma corticosterone levels were measured before and after 1 h strict immobilization. In control females, immobilization induced a 2.2-fold increase in corticosterone level on average, whereas, in NAF, the response to an acute stress was completely absent. One possible reason for this might be estrogen deficiency in females with polycystic ovaries. However, a more important pathogenetic mechanism is probably a violation of the hypothalamic neurotransmitter regulation that was confirmed in further experiments.

Anovulatory status caused by the injections of 150 mcg TP during PND 2-4, was characterized by a statistically significant decrease in the hypothalamic concentration of noradrenaline to $1.53 \pm 0.15 \times 10^{-9}$ g/mg tissue (control – $2.46 \pm 0.13 \times 10^{-9}$ g/mg tissue) and dopamine up to $1.46 \pm 0.37 \times 10^{-9}$ g/mg tissue (control – $3.35 \pm 0.31 \times 10^{-9}$ g/mg tissue). Serotonin concentration did not change. The histamine content increased to $1.25 \pm 0.10 \times 10^{-9}$ g/mg from $0.91 \pm 0.07 \times 10^{-9}$ g/mg in the control group, the acetylcholine concentration decreased to $0.68 \pm 0.07 \times 10^{-9}$ g/mg from $0.97 \pm 0.08 \times 10^{-9}$ g/mg in the control group.

Response of HPA axis to noradrenergic stimulation was studied in females aged 8 months. The experiments were carried out on unanaestetized, freely moving in the cage animals with a pre-installed metal cannula in the third brain ventricle and a Sylastic catheter in the external jugular vein. 10 mcg of noradrenaline bitartrate in 2 mcl of apyrogenic saline were infused for 1 min into the brain ventricle. Blood samples for spectrofluorometric assay of plasma corticosterone were collected before and after noradrenaline bitartrate infusion at intervals of 30 min, followed by replacement with an equal volume of heparin solution.

In control females, in response to noradrenergic stimulation corticosterone level grew 1.6 times on average after 30 min., then starting from 60 min it approached the baseline. In NAF, noradrenergic stimulation did not cause an increase in corticosterone levels at all.

A decrease in hypothalamic concentrations of noradrenaline and acetylcholine correlates with decrease in the content of hypophyseal gonadotropins and hypothalamic LH-RH in NAF, and an increase in histamine concentration correlates with an increased prolactin in adenohypophysis (apparently due to inhibi-

tion of secretion). Supposedly, catecholamine deficiency in the hypothalamus and HPA axis refractoriness to noradrenergic stimulation of the circumventricular structures are relevant pathogenetic mechanisms of impaired HPA response to an acute stress.

Our studies, similarly to ones by other researchers, have shown significant weakening of female sexual behavior and the presence of homo- and bisexual behavior induced by neonatal TP. In particular, the lordosis index in NAF who were ovariectomized and primed with estradiol and progesterone decreased ten times.

In search of early markers of SDB, we turned to proteins of neuroendocrine structures. Two proteins were found in the preoptic region, the contents of which differ in individuals of different sex. According to some data [16-17], the content of brain proteins with molecular masses of 53-56 kDa in neonatal rats can reflect sexual differences in the level of microtubulin, which is a marker of testosterone-induced neuronal growth. However, it remained unknown if the sexual features of the protein spectrum are due to genetic inheritance or whether hormones program them during perinatal development. Using disc electrophoresis in agar gel and densitometry, the spectrum of soluble proteins in the discrete brain areas involved in the neuroendocrine regulation of reproduction and HPA function was explored in our laboratory in normal males, females and NAF [18]. In normal rats, sex differences were found in a wide range of proteins mostly in the preoptic area (POA) of the hypothalamus, to a lesser extent in the mediobasal hypothalamus (MBH) at PND 5-10. In particular, the ratio of protein with a molecular weight of 66 kDa in the POA was 71% higher in males. Neonatal androgenization eliminated sex differences in the protein spectrum in this area by increasing the percentage optical density of the protein bands to a level that of normal males.

The most reliable early marker of impaired SDB in NAF is the changes in the steroid aromatase activity (AA) in the hypothalamus. This enzyme catalyzes the conversion of testosterone to estradiol and androstenedione to estrone. Testosterone induces aromatase synthesis in the hypothalamus. AA differs in male and female rats, especially in the POA, where it is higher in males [12, 19].

We studied in vitro conversion of [1,2,6,7-3H] testosterone to radiolabeled estradiol-17β and 5α-reduced metabolites in the supernatant (1000 g) of 10% brain tissue homogenate in the presence of a NADP·H-generating system in NAF and normal rats after isolation of steroids with two-dimensional thin-layer chromatography in Silicagel followed by β-radiometry [6]. In normal males, AA in the POA was three-five times higher than that of females, and the 5α-reductase activity, on the contrary, was significantly lower. Sex differences of 5α-reductase activity were not detected in either POA or MBA. Neonatally administered TP induced in females elevation of AA in the POA measured on 10th postnatal day with no significant changes in 5α-reductase activity in both areas studied.

According to some early reports from Japan and the USA, 5α-dihydrotestosterone and other non-aromatizable androgens being introduced parenterally or intracerebrally to newborn females were unable to program masculinization of developing brain. On the other hand, inhibition of aromatase prevented the brain defeminization in male rats [20]. We contributed to the hypothesis of mediating the role of estrogenic testosterone metabolites in SDB by studying the long-term effects of androgen receptor antagonists, flutamide and hydroxyflutamide, and steroid aromataze inhibitors, androst-1,4,6-trien-3,17-dion and androst-4-en-3,6,17-trion, on NAF. Both enzyme inhibitors reduced by 6 times the frequency of anovulation and persistent estrus caused by the injection of 50 mcg of TP on the PND 5 [21]. The blockade of androgen receptor signaling did not have a preventive effect that confirms the role of the conversion of testosterone to estrogens, which does not require the participation of the androgen receptor.

The next step in our research of the neurochemical mechanisms of androgen-dependent

SDB was elucidation of the participation of catechol estrogens, which are formed in the hypothalamus from monophenolic estrogens (estradiol, estrone) by hydroxylation at positions 2 or 4 of ring A in a steroid molecule. They are capable of binding to estrogen receptors and initiating signaling pathways. The content of catechol estrogens in the hypothalamus is several times higher than that of estradiol and estrone; as ovulation inducers, they are superior to estradiol.

It has been reported that the administration of 2-hydroxy- or 4-hydroxyestradiol-17β to newborn female rats reproduces the effects of neonatal androgenization on the development of anovulation [22]. We investigated the ability of 2-hydroxyestrone, as well as the isomers of catechol estradiol, 2- and 4-hydroxylated estradiol-17β, and 4-hydroxylated estradiol-17α, with systemic (subcutaneous) or intracerebral administration in doses of 5-25 mcg to newborn female rats to cause the brain defeminization. In contrast to the above authors, there was no disturbance of the estrous cycle and ovulation in adulthood in animals subjected to 2-hydroxylated estradiol-17β. Similar negative results were obtained in experiments with 2-hydroxyestrone and 4-hydroxylated estradiol-17α. Instead, 4-hydroxyestradiol-17β caused the same effect as TP. Probably the difference in the defeminizing activity of steroids is due to the known lower metabolic clearance and the much greater affinity of 4-hydroxyestradiol-17β to estrogen receptor as compared to other catechol estrogens.

One of the interesting phenomena is an increase in the content of noradrenaline in the whole brain of newborn female rats under the influence of testosterone [23]. We have found out a twofold increase in noradrenaline content in the hypothalamus of seven-day-old female rats to the level of normal males as a result of the administration of 250 mcg TP on PND 3 ($7.3 \pm 2.1 \times 10^{-9}$ g/mg tissue vs $2.6 \pm 0.1 \times 10^{-9}$ g/mg in controls, $P < 0.05$). Nevertheless, it remained unknown whether this phenomenon is relevant to SDB or to another testosterone effects. Dopamine content remained unchanged, and serotonin decreased slightly.

According to our research, the introduction of α-methyl-p-tyrosine, an inhibitor of catecholamine synthesis, prevented in newborn male rats the programming of hypothalamus refractoriness for the stimulating effect of estrogen on the regulation of LH secretion in adulthood. This evidenced by the appearance of luteal bodies in the fragments of ovaries of prepuberal females transplanted into the anterior chamber of experimental males [24]. Therefore, hypothalamic noradrenaline is critically important for the normal differentiation of the male hypothalamus, and its deficiency leads to the genetically determined brain programming by the female type.

In experiments on NAF, there were used alpha- and beta-blockers, presynaptic blockers of catecholamine and serotonin synthesis, catechol-O-methyltransferase and steroid aromatase inhibitors and other agents. TP at a dose of 25 mcg was injected on PND 3 or 4. At the same time, one of the neurotropic drugs was administered, and the treatment continued for a total of 5 days. Cyclic morphological changes in vaginal smears, ovarian morphology, concentrations of estradiol, progesterone, and LH in blood plasma were investigated at the age of 3 months.

α-Methyl-p-tyrosine was the most effective protector of anovulation syndrome. It preserved the proestrus surge of LH secretion and prevented cystic transformation of the ovaries. Another argument for the participation of noradrenaline in SDB was that an additional increase in the concentration of noradrenaline in the hypothalamus of NAF by giving them tropolone, a catechol-O-methyltransferase inhibitor (100 mcg from 4th to 10th day), increased the number of animals with anovulation syndrome from 70 to 100%, that is augmented defeminizing effect of TP [25]. However, tropolone-induced increase in noradrenaline content in the hypothalamus of non-androgenized neonatal females did not cause anovulation in adulthood. Therefore, noradrenaline alone is unable to direct the development of the neuroendocrine system along with

the male type; it is only effective in cooperation with steroids.

We were lucky to find out the neurochemical mechanism of increasing the concentration of hypothalamic noradrenaline under the influence of neonatal testosterone. Since the 70s of the last century, it was known that 2-hydroxyestradiol and 2-hydroxyestrone inhibit catechol-O-methyltransferase activity in the brain [26]. This enzyme metabolizes catecholamines and catechol estrogens by transferring a methyl group from S-adenosylmethionine to one of the phenolic hydroxyl groups. The consequence of the competition between catechol estrogens and noradrenaline for the active sites of catechol-O-methyltransferase is the inhibition of the conversion of noradrenaline to normethanephrine and an increase in the concentration of noradrenaline in tissues.

We hypothesized that 4-hydroxyestradiol-17β also has a similar property, and as a testosterone mediator in the process of SDB is responsible for the increase in hypothalamic noradrenaline level. This was confirmed by the results of studies, according to which steroid aromatase inhibitors prevented the rise of hypothalamic noradrenaline in NAF [27]. In addition, 24 h after subcutaneous injections of 4-hydroxyestradiol-17β to normal female rats at a daily dose of 10 mcg during the first postnatal days, the concentration of noradrenaline in the hypothalamus increased from $3.04 \pm 0.20 \times 10^{-9}$ mole/g tissue to $5.50 \pm 0.62 \times 10^{-9}$ mole/g tissue [11, 28]. The concentration of dopamine remained unchanged. Thus, it was proved that the increase in the concentration of noradrenaline in the hypothalamus under the influence of 4-hydroxyestradiol-17β is associated with defeminizing effect of the latter in females during the critical period of SDB.

Noticeably, the blockade of adrenergic receptors was not capable of interfering with testosterone to disrupt SDB. We have come to the conclusion that the participation of noradrenaline in SDB does not require synaptic transmission, that is, catecholamine in this case acts as a "pre-nerve" inducer of neurocytes differentiation.

The association between changes in noradrenaline levels in the hypothalamus of newborn rats and long-term consequences of SDB is presented in the diagram (Fig. 1). Alteration of the neurochemical phenotype of the brain as a consequence of programming caused by neonatal androgenization reflects the key mechanisms in the pathogenesis of male-type brain differentiation. Androgen-dependent SDB is the result of the cooperative action of catecholamines and neurosteroids, the last ones formed in the nervous tissue are estrogens and catechol estrogens. We have called this phenomenon a hormone-neurotransmitter con-induction that provides differentiation and functional maturation of immature neurocytes by epigenetic imprinting. We believe that estrogens (in particular, 4-hydroxyestradiol-17β) and noradrenaline are the substantial neurochemical determinants of androgen-dependent SDB [10, 29]. Estrogen involvement in SDB is necessary considering that estrogen receptor antagonists prevent testosterone-induced anovulation development as well as steroid aromatase inhibitors. A commonly recognized activity of estrogens is stimulation of synaptogenesis in neuroendocrine structures of the developing brain, which is essential for SDB.

Given the role of calcium ions in neurogenesis (neurocytes differentiation, migration, proliferation, apoptosis), it was considered appropriate to investigate their involvement in SDB. We studied the effects of neonatal administration of verapamil, a calcium L-type channel blocker, on the stress and noradrenergic responses of HPA axis in adult female rats with anovulatory syndrome induced by neonatal androgenization (250 mcg TP on PND 3). Although the pharmacodynamic effects of verapamil occur predominantly in the myocardium and blood vessels, it is able to penetrate the blood-brain barrier and block membrane calcium channels in the brain neurons [30].

There were no HPA axis responses to 1 h

immobilization or noradrenaline bitartrate (10 mcg) infusion in the third brain ventricle in females with persistent estrus. Verapamil treatment (50 mcg/100 g b.w., s/c for PND 3-7) preserved the rise in plasma corticosterone in both cases. These finding demonstrates the possible involvement of calcium signaling in the pathogenesis of the HPA disorders in NAF.

The proposed mechanisms of early differentiation of neuroendocrine control of gonadotropin secretion, sexual behavior, and HPA axis function in NAF are shown in the scheme (Fig. 2).

Prenatal glucocorticoid effects on developing brain

Hydrocortisone. The developing brain is characterized by extremely high plasticity and is very sensitive to changes in hormonal balance. Glucocorticoids of maternal or exogenous origin easily cross the placental barrier and play an important role in the differentiation and programming of fetal neurons [31, 32].

According to numerous animal research, prenatal glucocorticoids cause a variety of behavioral disorders in adult life, modification of the basal level of corticosteroids, HPA axis reactions to stress stimuli, violation of reciprocal relationships between the adrenal glands and the hypothalamic-pituitary complex. The latter is associated with a decrease in the expression of corticoliberin mRNA in the hypothalamus, and with a change in the density of corticosteroid receptor distribution in the brain, in particular, in the hippocampus [31]. One of the possible mechanisms of the programming effect of glucocorticoids on the developing brain is inhibition of the neurocytes apoptosis. From a practical point of view, it is of importance that the administration of glucocorticoids in the perinatal period is associated with long-term adverse effects on neurodevelopment [33].

Our studies in this field concerned the effects of prenatal glucocorticoids on Wistar rats. Although corticosterone is the main glucocorticoid in rats, hydrocortisone was used in the experiments, because it is very close to corticosterone in terms of hormonal activities.

Suspension of hydrocortisone acetate (HA)

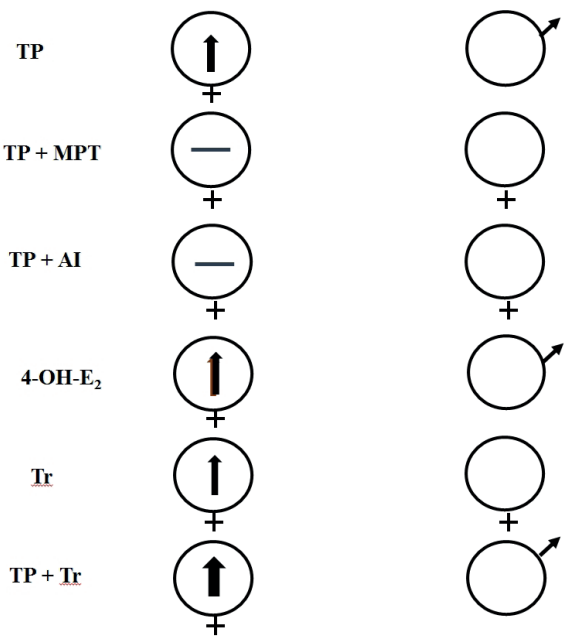

Fig. 1. Dependence of the sex brain differentiation on changes in the concentration of noradrenaline in the hypothalamus of female rats on the 7th day after birth (shown with the arrows).
Abbreviations: TP – TP; MPT – α-methyl-p-tyrosine; AI – aromatase inhibitor; 4-OH-E2 – 4-hydroxyestradiol-17β; Tr – tropolone

was administered subcutaneously to pregnant females at a daily dose of 50 mg/kg b.w. on gestational days 15-21 or 16-18. Due to the hormone supplementation during the 15-21th day of pregnancy, the level of 11-hydroxycorticosteroids (hydrocortisone plus corticosterone) in blood plasma on the 21st day increased by an average of 53%. Early and long-term postnatal effects of prenatal HA on sex-related peculiarities of the brain morphology, biogenic monoamine contents and turnover, testosterone metabolism, adrenocortical responses to an acute stress and noradrenergic reactivity of HPA axis were studied [12, 34-36].

Disorders of SDB have been revealed in the next few days after birth. The sex differences of AA in the POA on PND 10 were absent because of an increase in enzyme activity in female offspring. In the MBH, aromatase sexual dimorphism was absent in both control and experimental animals, although AA in the experimental females was significantly reduced compared to the corresponding control.

Prenatal HA impaired the formation of 5α-reduced metabolites of testosterone in the MBH. Under normal conditions, the activity of 5α-reductase (measured as the sum of 5α-dihydrotestosterone and 5α-androstan-3α,17β-diol, formed in vitro from testosterone for 1 h per g of protein) in the POA of females was higher than that of males (on average, 329 U vs 212 U, $P < 0.05$ by the nonparametric criterion U). In experimental rats, this difference disappeared (females - 237 U, males - 190 U protein, $P > 0.05$). The concentration of dopamine in the POA did not change.

Glucocorticoid treatment eliminated the sex difference in noradrenaline concentrations in the POA of 10-day-old offspring due to a decrease in females (control: females, $4.22 \pm 0.48 \times 10^{-9}$ mole/g tissue; males, $2.74 \pm 0.34 \times 10^{-9}$ mole/g tissue, $P < 0.05$; HA: females, $2.64 \pm 0.37 \times 10^{-9}$ mole/g tissue; males, $2.16 \pm 0.31 \times 10^{-9}$ mole g tissue, $P > 0.05$). Probably, this phenomenon relates to the programming inhibitory effect of prenatal HA on the rat hypothalamic activity of tyrosine hydroxylase, a limiting enzyme in catecholamine biosynthesis, in the fetal brain [37].

In the study of the rate of catecholamine turnover in the discrete structures of the hypothalamus of 10-day-old rats there was used tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine, which was injected subcutaneously 1 h or 2 h before decapitation of animals. Catecholamine

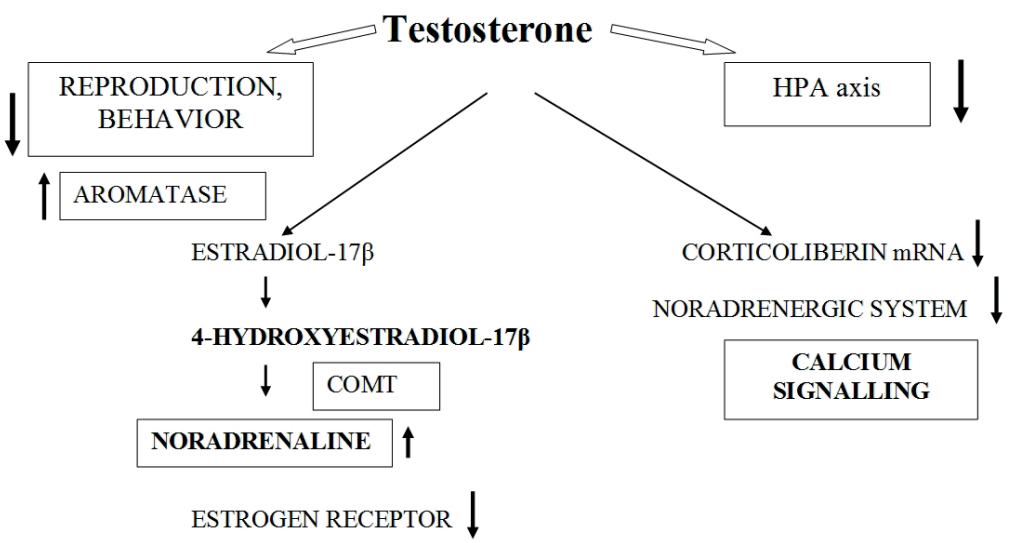

Fig. 2. Neurochemical determinants of the sex brain differentiation in neonatally androgenized female rat.
Abbreviations: HPA axis – hypothalamic-pituitary-adrenal axis; COMT – catechol-O-methyltransferase

turnover rates were calculated according to Brodie et al. [38]. It is known that a change in turnover rate during the first hour after injection reflects the velocity of using the functional catecholamine pool.

Prenatal HA led to a significant decrease in noradrenaline turnover in the male POA (0.33 ± 0.09 × 10^{-9} mole/g·h vs 1.89 ± 0.45 × 10^{-9} mole/g·h in control males, $P < 0.05$) and an increase in the dopamine turnover rate in females (0.69 ± 0.12 × 10^{-9} mole/g·h vst 0.33 ± 0.11 × 10^{-9} mole/g·h in control females, $P < 0.05$). There were no changes in these values in the MBH of the experimental animals.

No sex differences were detected in serotonin and 5-hydroxyindolacetic acid levels assayed by high-performance liquid chromatography in both areas of the hypothalamus of normal rats. Prenatal HA caused the appearance of sex differences in the concentration of 5-hydroxyindolacetic acid in both POA and MBH and serotonin metabolism – in the POA due to an increase in these parameters in females. The concentration of serotonin in the POA was higher in the experimental males.

Prenatal HA affected the characteristics of SDB and HPA axis function in adulthood (6-8 months). Samples from experimental and control females were collected in the diestrus stage.

According to our research, a neuromorphological marker of HA-induced SDB disorders was the disappearance of sexual dimorphism of the neurocyte nuclei size in medial preoptic (MPN) and suprachiasmatic nuclei of the hypothalamus. In normal rats, it is 20-25% higher in males compared to females. These differences are attenuated in the suprachiasmatic nuclei because of reducing those values to that of normal females. In MPN, sexual dimorphism of karyometric indices also disappeared, but by increasing the size in females. These changes correlate with disorders of sexual behavior and fertility in male rats prenatally exposed to HA [39].

It has been confirmed that HPA response to 1 h immobilization weakened in adult male offspring prenatally exposed to HA. This is probably due to the modification of neurotransmitter regulatory systems. HA-exposed males lacked stress-specific decline in the concentration of noradrenaline in the hypothalamus and increased activity in the hippocampal glutamate decarboxylase, which is a limiting enzyme in the synthesis of GABA. In contrast to the males, the experimental females responded to an acute stress with a more expressed elevation of corticosterone level relative to controls associated with a more pronounced decrease in hypothalamic noradrenaline concentration and an increase in glutamate decarboxylase activity in the hippocampus.

On unanesthetized and unrestricted adult females and males, we have studied the HPA response to corticotropic or central noradrenergic stimulation (10 mcg of noradrenaline bitartrate into the 3rd brain ventricle). In contrast to the control males, the HA-exposed animals did not experience corticosterone rise in blood plasma during the 90-minute observation. This did not related to changes in the adrenal cortex, because of maintaining its sensitivity to β-1-24-corticotropin intravenous infusion either in males or in females.

Thus, induced by prenatal HA changes in the functional state of neuroendocrine system depend on sex.

Dexamethasone. One of the widely used glucocorticoid drugs is the synthetic steroid, dexamethasone. It binds the glucocorticoid receptor with high affinity and almost not metabolized by placental 11-beta-hydroxysteroid dehydrogenase. It crosses the placental barrier freely into the fetal circulatory system and is relatively easy to overcome the fetal blood-brain barrier.

Prenatal administration of dexamethasone led to sexual behavior abnormalities in adult male rat offspring [40, 41]. This preceded by a decrease in AA (by an average of 45%) in the POA of 10-day-old rats. Under the influence of dexamethasone injections (100 mcg/kg daily during the 15-21st days of pregnancy), sex dif-

ferences in the rate of metabolic conversion of testosterone to estradiol disappeared; however, there were no changes in AA in the MBH [42]. There was found out an inversion of sex differences in the formation of 5α-reduced metabolites of testosterone in the POA and the MBH due to decreased 5α-reductase activity in females.

Changes in HPA axis response to an acute stress in terms of plasma corticosterone levels and hypothalamic noradrenaline concentrations and turnover in adult males and females were the same as those under prenatal HA [43]. Analysis of the $GABA_B$ receptor as a participant of stress-limiting mechanism in the central nervous system was carried out using this receptor agonist, baclofen, which was introduced 30 min before 1 h immobilization intraperitoneally at a dose of 10 mg/kg BW. In control males, baclofen caused a limitation of corticosterone response to an acute stress. Instead, prenatally exposed to dexamethasone animals did not show any stress-response after baclofen premedication. It seems that prenatal dexamethasone damages in males the programming of the stress-limiting $GABA_B$ signaling. In females exposed prenatally to dexamethasone, the stress-induced HPA reaction on the background of the activation of $GABA_B$ receptor persisted: administration of baclofen prior stress restricted the hormonal response similarly to control animals. Thus, alteration of the HPA axis function with prenatal dexamethasone demonstrates distinct sex differences.

Pharmacological analysis of mechanisms of developing brain programming

GABA agonists and antagonists. One of the most commonly used GABA agonists is phenybut (β-phenyl-γ-aminobutyric acid), which has anxiolytic, tranquilizing, sedative and anti-stress effects. The study of its effects in the aspect of functional teratology was carried out in the offspring of rats whose mothers were given the drug (phenybut) by gastric intubation during the last week of pregnancy at a daily dose of 100 mg/kg b.w. [44, 45].

The testicle descent into the scrotum of control males occurred on PND 38.5 ± 0.1, while in phenybut group it accelerated by 4.7 days (33.8 ± 0.1, $P < 0.001$), which indicates puberty praecox. Females also revealed premature puberty – the date of vagina opening diminished by an average of 4.5 days compared with control animals (respectively, 39.3±1.0 and 43.8 ± 0.2 days, $P < 0.001$).

In adult males, the numbers of mounts with intromission in the first and second tests reduced by third compared to intact controls, indicating a decrease in male sexual performance. Testing of female sexual behavior of males revealed lordosis reactions to the presence of receptive female, that is, a feminizing effect.

In females, the female-type sexual behavior did not change, but male behavior was characterized in all animals by mounts on receptive female, that is, quasi-copulatory homosexual behavior.

In adult males prenatally exposed to phenybut, plasma corticosterone response to an acute stress (1h restriction) doubled. Therefore, GABAergic regulation is of importance for the normal development of the fetal nervous system that regulates sexual behaviors and HPA axis function.

Drugs that affect the synthesis, metabolism and reception of catecholamines. Experimental evidence that drugs whose pharmacological target is the brain catecholaminergic system disrupt androgen-dependent SDB was obtained in different animal species in the second half of the last century. In our laboratory, there were investigated the prenatal effects of methyldopa (400 mg/kg/day during the 15-21st days of pregnancy) on the sexual behavior [44] and HPA function [45] in the male rat progeny aged 3 months. This medicine overcomes the blood-brain barrier and is used in pregnant women as a relatively safe antihypertensive drug.

In naïve male offspring, prenatal methyldopa more than doubled latency to the first mount (91.0 ± 18.7 vs 37.2 ± 4.9 sec in control on av-

erage, $P < 0.05$) and first intromission (93.0 ± 17.6 vs 40.4 ± 5.3, $P < 0.05$) in the presence of receptive female. In one week, with the acquisition of sexual experience, latency and the total number of mounts did not change, however, the number without intromission decreased twice.

The ability to sexual behavior by female type was investigated on castrated and pre-treated with estradiol diacetate and progesterone males in the presence of a normal male. In contrast to normal males, they displayed lordosis behavior, and some of them performed mounts on normal males.

The introduction of methyldopa to pregnant dams had a certain modifying effect on the sexual characteristics of HPA axis response to an acute stress in their adult offspring. Males showed a moderate increase in the stress responsiveness, while in females, the amplitude of the post-stress increase in the corticosterone level was significantly less than that in controls. It is known that under normal conditions, the stress reactivity of the HPA in female rats is higher than that in males. Therefore, its changes induced by prenatal methyldopa occur in the direction of opposite sex.

Thus, prenatal intervention in catecholaminergic system of the male brain causes feminization and partial demasculinization of reproductive behaviors in adulthood, as well as mitigation of sex differences in the HPA axis function.

Blockade of calcium ion channels. Above are the results of the study of the effect of verapamil on NAF. In continuation of this work, it was considered appropriate to explore the participation of calcium signaling in the programming of brain development under normal conditions [46, 47]. Rats were treated with nimodipine (20 mg/kg BW/day intragastrically) suspended in a Dorfman gel at 15-21st days of gestation. This dosage is optimal for blocking L-type calcium channels in brain cells [48].

Prenatal nimodipine resulted on PND 5 in an increase in the relative content of soluble cytosolic proteins of 66.0 and 34.7 kDa (by 22 and 43%, respectively, $P < 0.05$) in the POA of female hypothalamus compared with intact females, which brought these measures closer to intact males. In the male group, there was a decrease in the relative content of proteins of 66.0 and 18.4 kDa (by 35 and 21%, respectively, $P < 0.05$).

An early sign of androgen-dependent SDB programming under the influence of prenatal nimodipine was a nearly twofold decrease in AA and an increase in steroid 5α-reductase activity in the POA of 10-day-old males. In females of that age, the activity of 5α-reductase increased in both POA and MBH.

In the female progeny, nimodipine caused delayed puberty (on average by 10 days) in 20% of the animals. The phase structure of estrous cycles changed with lengthening diestrus. This may be due to early-life changes in hypothalamic 5α-reductase activity. The female sexual behavior remained unchanged. At the same time, their heterotypic sexual behavior significantly changed. They all showed features of male sexual behavior in the presence of receptive female in the form of mounts with average of 7.0 ± 1.5 for 15 min of testing, which were absent in normal female. Besides, nimodipine-exposed females displayed extremely active proceptive behavior: the number of approximations to the receptive female increased significantly from the control values of 5.8 ± 1.1 to 14.0 ± 1.5 ($P < 0.01$), which indicates the masculinizing effect of prenatal nimodipine.

In adult male rats, prenatal nimodipine did not exert a significant effect on the formation of male sexual behavior, while signs of female sexual behavior emerged in castrated animals pre-treated with estradiol and progesterone. The number of males with female receptive behavior (lordosis) significantly increased. 80% of experimental males showed active homosexual behavior, 60% displayed bisexual behavior. Apparently, these violations reflect significant changes in the behavioral response to the activation by female sex hormones, in contrast to

normal males, which are characterized by refractoriness to them. We concluded that prenatal blockade of calcium signaling with nimodipine in the critical period of SDB causes feminization of sexual behavior in males and masculinization in females.

No pronounced changes in the reaction of HPA axis to 1 h immobilization were detected in nimodipine-exposed males and females, though the noradrenergic stimulation of the circumventricular structures of the hypothalamus was prolonged in both them.

The findings of research with nimodipine indicate involvement of calcium signaling in the programming of neuroendocrine system of developing brain.Given together, the above presented examples of adverse long-term effects on the offspring of the use of some drugs during pregnancy might be considered in obstetric practice to evaluate their potential risk to the fetus development.

Prenatal stress syndrome

Prenatal stress syndrome was discovered and named in 1972 by I. Ward 1972 [49]. PS males exhibited low copulatory activity (21% of animals mated and ejaculated vs 64% in the control group) and high rates of lordosis, that is, female sexual behavior. The author associated the identified changes with a stress-induced reduction in fetal testicular testosterone secretion during the critical stage of SDB. G. Dörner and co-workers were the first to obtain evidences that PS syndrome is present in real human life [50, 51]. Environmental pollution, social turbulence, wars, technogenic accidents, fear of losing a job, physical violence, family disputes can all cause severe stress (distress).

Later in various laboratories, it was shown that similar disorders occur in other species of animals, as well as with other methods of stressing - pain, cold, fasting, emotional factors and so on. The accumulation of facts about the negative impact of gestational stress on offspring continues in various countries, such as Israel, where populations are exposed to rocket attacks, Canada, where natural disasters occur, and so on. The concept of PS syndrome expanded significantly when it became clear that it causes alteration not of sexual behavior only, but also other numerical abnormalities: disorders of lipid and carbohydrate metabolism, changes in stress reactivity of HPA axis, subfertility of females, oxidative stress, etc. in both sexes. Stressing rats at an early postnatal life has similar consequences, and this is due to the fact that SDB has not yet completed during this period.

For many years, our team studied the pathogenesis of PS syndrome and the possibilities of its prevention [7, 12, 34, 44-47, 52-61]. The experimental model was the daily one-hour immobilization of rats in the last week of pregnancy. The effects of PS were examined in rats aged 3, 6-8, 19-28 months.

Below is a list of phenotypic features of PS syndrome. The results of our research are marked with an asterisk.

Male rats

• Homo- or bisexual behavior, decrease in the number of mounts, intromissions, ejaculations, increase of their latent periods *

• Increasing aggressive behavior

• Reduction of the size of the sex-dimorphic nucleus of the anterior hypothalamic region

• Reduction of the volume of neuron nuclei in the MPN and suprachiasmatic nuclei of the hypothalamus *

• Early changes in the concentrations and metabolic turnover of catecholamines in the brain*

• Early decline of testosterone conversion to estradiol-17β in the POA *

• Early disorders of testosterone conversion to 5-alpha-dihydrotestosterone and other 5-alpha-reduced metabolites in the MBH *

• Puberty delay *

• Suppression of pituitary LH response to LH releasing hormone, aromatase inhibitors and androgen receptor antagonists *

• Suppression of pituitary ACTH response to arginine-vasopressin *

• Acceleration of age-related involution of

spermatogenesis *

• Reduction of the response of the adrenal cortex to an acute stress *

• Changes in the number of glucocorticoid receptors in the hippocampus

• Delayed degranulation of hypophyseal corticotropocytes in response to an acute stress *

• Insufficient release and utilization of catecholamines in the hypothalamus in an acute stress *

• Disruption of serotonergic control of HPA axis response to an acute stress *

• HPA axis nordarenergic hypersensitivity *

• Activation of the brain stress-limiting systems (GABA *, atrial natriuretic peptide, melatonin, beta-endorphin*, PG 2, prostacyclin, etc.)

• Disappearance of early sex differences in certain protein in discrete brain regions*

• Violation of lipid and protein peroxidation in the brain

• Decrease in the activity of microsomal monooxygenases in the liver *

Female rats

• Puberty delay *

• Changes in the structure of estrous cycles *

• Decrease in fertility potential and fecundity

• Moderate increase in adrenal cortex response to an acute stress *

• Reduced physical endurance *

• Reduction of the HPA axis noradrenergic sensitivity *

• Increase in the activity of microsomal monooxygenases in the liver *

From this list, it is obvious that the fetal male brain is much more sensitive than in females to the damaging effect of PS. It is proved that the main factor of demasculinization/feminization of the male brain is transient deficiency of testicular testosterone. This is justified by the preventive effect of perinatal use of testosterone on the demasculinization and feminization of sexual behavior of adult males [62] and on testosterone aromatization and protein pattern in the POA of newborns [59] resulting from PS for the last gestational week. Impairment of brain programming in females is probably due to an increase in the secretion of androstenedione by maternal adrenals and its penetration into the fetus's blood circulation.

Changes in the neurochemical determinants of SDB in PS males have been revealed mainly in the POA, the most important locus of androgen-dependent sexual programming of the developing brain. AA in 10-day-old males was almost halved ($0.38 \pm 0.05 \times 10^{-12}$ mole/g·h tissue vs. $0.62 \pm 0{,}07 \times 10^{-12}$ mole/g·h tissue in control), while in females it did not change.

Sexual differences in the content of noradrenaline in the rat hypothalamus develop during the maturation of the brain catecholaminergic system on the PND 7 and reach a statistical significance on the PND 10. Therefore, we conducted appropriate studies on 10-day-old rats.

Under normal conditions, the concentration of noradrenaline in the POA of males is almost half that of females. This corresponds to a more accelerated metabolic turnover of noradrenaline in males, which we studied using α-methyl-p-tyrosine for blockage of catecholamine synthesis. Sexual differences in the content and circulation of noradrenaline in the male POA disappeared due to their approximation to these parameters in females. In particular, noradrenaline level grew from $2.9 \pm 0.2 \times 10^{-9}$ mol/g tissue to $4.7 \pm 0.6 \times 10^{-9}$ mol/g tissue ($P < 0.05$), while it did not change in females (control – $4.4 \pm 0.6 \times 10^{-9}$ mol/g tissue, PS – $3.5 \pm 0.4 \times 10^{-9}$ mol/g tissue, $P > 0.05$). PS did not change dopamine and serotonin levels in the POA of males.

Changes in soluble protein spectrum also reflect the disorders of SDB after PS. PS eliminated sexual differences in the relative content of 66.0 kDa protein and at the same time caused them to appear in 18.4 kDa protein in the POA. Although under normal conditions the sex difference of the studied protein spectrum was not observed in the MBH and hippocampus, it appeared as a result of PS.

Sex-dimorphic formations of the hypotha-

lamus also include suprachiasmatic nuclei. The karyometry of the neurocytes of these nuclei in 10-day-old PS males demonstrates a change in SDB toward demasculinization. The mean volume of nuclei of neurons in males averaged 233 ± 7 μm^3 vs 193 ± 10 μm^3 in females ($P < 0.05$). This difference disappeared in the PS rats due to the nuclei volume reduction in males to 189 ± 7 μm^3, while maintaining the volume of 189 ± 21 μm^3 in females. A similar effect of PS was also seen in 3-month-old rats.

Early neurochemical and microstructural changes in the male hypothalamus are predictors of neuroendocrine and behavioral abnormalities in adulthood. PS prolonged latency of the first mount and intromission, and post-ejaculatory refractory period twice, significantly reduced the number of ejaculations. All PS males who were castrated and primed with estradiol and progesterone displayed lordosis reactions in the presence of normal males.

The weakening of the HPA response to one-hour restriction in 3-month-old males was associated with the absence of a stress-induced decrease in noradrenaline concentration in the hypothalamus. Instead, the response of HPA axis to central noradrenergic stimulation lengthened in time. Plasma corticosterone response to β-1-24-corticotropin stimulation in non-anesthetized males did not change, as well as in experiments with stimulation of corticosterone secretion with corticotropin in vitro by rat adrenal slices. Alterations in the HPA axis were not detected in 3-month-old PS females, although at the age of 18-21 months, corticosterone response to an acute stress was insufficient when compared with controls of the same age.

The state of GABA receptors as a part of a stress-limiting system was analyzed in adult PS rats using $GABA_A$ receptor agonist, muscimol, and $GABA_B$ receptor agonist, baclofen, which were administered intraperitoneally at a dose of 0.1 and 10 mg/kg, respectively, 30 min before immobilization. Under normal conditions, they respond to an acute stress by reducing the plasma corticosterone response. In prenatally stressed adult males, there is a refractoriness of $GABA_A$ and $GABA_B$ receptors to their activation, possibly as a manifestation of compensation for catecholaminergic deficiency.

In order to address the ways and mechanisms of realization of pathogenic effect of maternal stress on the programming of neuroendocrine functions in intrauterine fetus, various pharmacological preparations were used.

Blockade of the HPA axis stress response with dexamethasone (s/c 100 mcg/kg, 30 min before one-hour immobilization of females during 15-21 days of pregnancy) prevented early and long-term negative neurochemical, behavioral, and other PS effects in male progeny. This indicates initiating role of glucocorticoids and their receptors as mediators in the pathogenesis of PS syndrome.

One of the approaches to pharmacological blockade of maternal stress is stimulation of the brain's stress-limiting system. Prenatal use of the GABA agonist, phenibut (100 mg/kg, per os), according to the same scheme as dexamethasone, prevented the development of early postnatal changes in AA in the POA of males and significantly improved the timing of puberty. Prenatal phenibut completely normalized male sexual performance in male offspring, reduced by a third the number of lordosis reactions, restored the HPA axis response to an acute stress, but did not affect its noradrenergic sensitivity.

According to the literature [63], sexual dysfunction in PS male rats can be prevented by administering to pregnant mothers tyrosine, the precursor of catecholamine synthesis. The preventive effect of tyrosine is associated with the restoration of noradrenaline content in the hypothalamus, which reduced due to stress. These findings are consistent with the concept of the involvement of noradrenaline in SDB as its important determinant.

In order to clarify the role of maternal noradrenergic activation as a part of stress in association with SDB disorders, we used methyldopa as sympatholytic agent (400 mg/kg, per os, 30 min before immobilizing procedure).

Prenatal methyldopa contributed to the partial normalization of AA in the POA of males at PND 10, some indices of male sexual behavior, but did not prevent lordosis reactions and did not affect the PS-induced modification of the HPA axis stress reactivity.

Opioids are an important component of the stress-limiting system of the brain. Stress in pregnant rats is accompanied by an increase in the content of opioids, in particular, β-endorphin, in the hypothalamus and adenohypophysis of the mother and fetus [64]. Signs of the failure of the SDB in adult male rats are expressed after the introduction of β-endorphin at a dose of 33 mcg three times a day to their mothers in late pregnancy [65]. In our laboratory, it was shown that the introduction of β-endorphin in a similar scheme reproduces the effect of PS on AA in the POA [61]. Opioids have been suggested to be responsible for inhibiting testosterone secretion by fetal testicles during stress due to a decrease in hypothalamic LH-RH secretion and, consequently, for demasculinization of the brain. It has previously been found out that the use of naltrexone, an opioid antagonist, which mainly binds to mu-receptors, during stress in pregnant dams, prevents impairment of sexual behavior in male offspring [66]. However, the neurochemical basis of this effect remained unstudied.

In our research, naltrexone pre-treatment of stressed dams (10 mg/kg, s/c, daily, 30 min before one-hour immobilizing during the last week of pregnancy) prevented modifying the effect of PS on AA, noradrenaline content, and protein pattern in the POA of 10-day-old males. In adulthood, they demonstrated normal sexual behavior and HPA axis function including corticosterone and hypothalamic noradrenaline response to an acute stress and baclofen, as well as HPA axis noradrenergic sensitivity. These findings indicate that endogenic opioids mediate detrimental effect of PS on neurochemical determinants of the SDB that may underlie demasculinization of the male sexual behavior.

Calcium is one of the most important ions required for brain development and function including interneuronal signaling, synapse formation, transmission and plasticity, and apoptosis and necrosis. The role of calcium signaling in the pathogenesis of PS syndrome was studied by stressing pregnant rats on the background of nimodipine, which was introduced orally at a dose of 20 mg/kg 30 min before immobilization. At this dose, nimodipine blocks L-type channels in brain cells [48]. Sexual dimorphism of AA and 66 kDa protein content in the POA of 10-day-old PS rats disappeared due to their drop in males.

Prenatal nimodipine did not affect PS-induced delayed puberty in males and females. At the end of the 6-month follow-up period in females, changes in estrus cycles were less pronounced, instead, they maintained normal female behavior.

Prenatal nimodipine normalized absolutely all studied indicators of male sexual behavior in PS males and prevented modification of HPA axis stress-responses in males and females. Given together, these data indicate an important role of calcium signaling in the pathogenesis of PS syndrome.

Endocrine disrupting chemicals and SDB

The impact of endocrine disrupting chemicals (EDC) as environmental pollutants on the developing brain organization has been in the circle of scientific interest of G. Dörner and his colleagues [67]. Today, the impact of EDC on human health and especially on pregnant mothers and fetuses are the focus of research around the world [68, 69]. A distinctive feature of EDC is the lack of dependence of the pathogenic effect on the dose of exposure, which complicates the establishment of threshold toxic doses and the predictability of the consequences. EDC interferes with one or simultaneously with several biological processes involved in hormonal regulation [70]. Agonistic or antagonistic effect on natural hormonal ligands is realized through interaction with estrogen, androgen, progesterone receptors, which leads to disorders of intra-

cellular signaling mechanisms and functioning of genome. In addition, they are capable of causing oxidative stress.

The reproductive system is the most vulnerable to EDC [71-73]. The action of such substances on the pregnant woman causes a cascade of neurohormonal changes in the mother and fetus, which, with the involvement of imprinting mechanisms, program disorders of the neuroendocrine control of many physiological functions, including behavior, reproduction processes, and adaptation to changing environment. Moreover, under certain conditions, they cause teratogenic effects, such as cryptorchidism and gonadal dysgenesis.

Dibutyl phthalate. One of the most common endocrine disruptors are phthalic acid esters (phthalates). They are used mainly in manufacturing polyvinyl chloride products, for example, flooring and roofing, packaging for beverages, coating drugs and children's toys. Due to their inherent anti-androgenic activity, phthalates disrupt the normal development of the male reproductive tract. This effect is caused by inhibition of testosterone synthesis in fetal testicles and disorganization of the histoarchitectural structure of Leydig cells.

The long-term effects of pre- and perinatal administration of phthalates, especially dibutyl phthalate (DBP), on the fetus in terms of functional teratology are poorly studied. Published sources reported diminution of sexual behavior in male rat offspring. Unlike most of the published papers, in which the effects of DBP have been studied after its administration during embryo-, fetogenesis, and lactation, we have selectively investigated the epigenetic reprogramming of developing neuroendocrine system and the behavior that occurs at SDB window.

The minimum daily dose that did not cause any visible anatomical defects in male offspring of rats (NOAEL) when DBP was given to mothers during 12-20 days of pregnancy was set at 66 mg/kg/day [74]. We studied early and long-term consequences of prenatal low dose DBP (100 mg/kg/day by gavage for gestational days 15-21) on sexual maturation and behavior of Wistar male rat progeny [75]. At PND 2, the anogenital distance slightly reduced relative to the control, which is a marker of the antiandrogenic activity of DBP. Nevertheless, on the 7th day, this difference disappeared due to the completion of the crotch fusion. There were no other anatomical abnormalities in the newborns.

Surprising findings were that exposure of pregnant dams to low dose DBP during the critical period of the fetus SDB leads in male offspring to premature puberty, hyperandrogenism, and hyperactive male sexual behavior in reproductive age. The descent of the testes into the scrotum, which is an indicator of puberty, occurred in DBP group earlier compared to the control group. In six-month-old males, the level of testosterone in the blood plasma doubled ($7.53 \pm 1.45 \times 10^{-9}$ g/ml vs. $3.76 \pm 0.85 \times 10^{-9}$ g/ml in the control group, $P < 0.05$). It is likely that premature puberty is due to the hyperandrogenic condition of the animals.

Histological examination of the gonads revealed no changes in the spermatogenic layer of seminal tubules. Numerous Leydig cells were enlarged and in the active synthesis phase, which explains the increase in plasma testosterone levels. The sperm concentration in the flushes of the epididymis increased slightly.

Testing of male sexual behavior in experimental animals at 6 and 10 months of age revealed hypersexual male activity toward receptive females. The latency of the first mounting, the first intromission, the first ejaculation, and the duration of the post-ejaculation refractory period significantly reduced. The number of intromissions and ejaculations increased. We call this phenomenon "prenatal dibutyl phthalate syndrome". If this phenomenon is reproduced in men in real life, it might be a pathogenetic basis of so-called criminal hypersexuality, which is usually associated with excessively high levels of testosterone in the blood.

In 18-month-old DBP-exposed rats, premature involution of testes and accessory sexual

glands as well as exhausting of male sexual potency occur. Testosterone levels in the blood plasma decreased by an average of two and a half time against corresponding aging controls ($1.04 \pm 0.17 \times 10^{-9}$ g/ml vs $2.48 \pm 0.71 \times 10^{-9}$ g/ml). The sperm concentration in the flushing of the epididymis decreased from $53.2 \pm 2.9 \times 10^6$/ml to $38.3 \pm 4.6 \times 10^6$/ml. The difference between the latency of the first mounting in the experimental and control groups disappeared, the number of intromissions reduced tenfold, and ejaculations were absent at all.

The hypersexual behavior of young males is probably due to excessive brain masculinization and high plasma testosterone levels. The hyperactivity of the neuroendocrine center of male sexual behavior is evidenced by the morphological signs of enhanced functional activity of neurocytes of the hypothalamic MPN. Presumably, the cause of excessive masculinization of the developing brain is increased synthesis of testosterone in the fetus testicles. The basis for this hypothesis is the information about the direct stimulating effect of low concentrations of DBP and its metabolite, monobutyl phthalate, on testosterone synthesis in mouse Leydig cell (MLTC-1) culture [76]. Instead, at high concentrations, both compounds inhibited steroidogenesis [77]. Our hypothesis relies on the data on the kinetics of testosterone secretion by fetal testicles resulting from feeding female rats at a daily dose of 100 mg/kg for gestation days 12-19 [78]. Within 24 h after inhibition of testosterone synthesis, plasma testosterone level restores and then grows above initial level due to rebound effect. A transient burst of testosterone secretion in the SDB window could be the cause of the brain hypermasculinization.

One of the consequences of prenatal low dose DBP was enhanced feminization of the male brain, which, in conditions of estrogen-progestin loading, manifested by an increase in the frequency of lordosis responses to the presence of a normal sexually active male. Both ten-month-old and aging males, castrated and hormone-primed, demonstrated a homosexual type of behavior. Unexpected activation of female sexual behavior might be caused by impaired defeminization of neuroendocrine structures, for example, because of DBP-induced oxidative stress.

In adult female offspring, we found quasi-copulatory behavior in the form of mounts on receptive females, although their lordosis behavior did not alter.

Bisphenol A. Bisphenol A (BPA) acts on the endocrine system as an estrogen receptor agonist. In our study, it was introduced into the stomach to pregnant Wistar rats during the last gestation week at a daily dose of 25 mcg/kg, which did not cause teratogenic effects in the descendants. Sexual behavior was examined in male descendants at the age of 10 months. Almost complete inhibition of copulatory components of male sexual behavior was observed against the background of normal testosterone levels and the absence of morphological changes of the gonads and prostate. According to histological and karyometric examination of the MPN, neurocyte activity was attenuated, correlating with inhibition of male sexual behavior.

One of the effects of prenatal BPA was the appearance of lordosis in ovaryectomized and primed with estradiol and progesterone males in the presence of a normal male, that is, conservation of the sensitivity of the neuroendocrine centers of reproduction to stimulation by female sex hormones in adult male offspring, indicating a severe violation of androgen-dependent SDB. Probably, the violation of androgen-dependent SDM is due to the antagonistic action of BPA against testicular testosterone of the fetus because of the estrogen-like activity of the disruptor.

Finally, it should be noted that disorders of early programming of neuroendocrine functions and behavior are polyetiological and polypathogenetic in nature and require further study.

CONCLUSIONS

Man is a part of the world of mammals, and the laws of human ontogenesis are largely common to other animal species. Therefore, there is a high probability that the results of animal studies could be extrapolated to human beings. The findings of experimental studies obtained in the last decades as well as clinical observations on the etiology and pathogenesis of psychological, neuroendocrine, and other functional disorders caused by numerous perinatal factors created a basis for the practical implementation of prevention of these disorders. Early-life programming of sexual behavior might explain the way of formation of gender self-identification. Epigenetic mechanisms of developmental modifications of neuroendocrine and behavioral individual phenotype need to be investigated in the future. Preclinical studies of new drugs intended for use in pregnant women should be complemented by an exploration of the long-term effects on fetal health. Psychological, somatic, and social aspects of early brain programming should be studied in the age aspect on an interdisciplinary basis. This will contribute to a deeper understanding of the biological and social nature of man, as well as the preservation of his health and quality of life.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study.

О. Г. Резніков

ФОРМУВАННЯ НЕЙРОЕНДОКРИННОЇ РЕГУЛЯЦІЇ РЕПРОДУКЦІЇ ТА АДАПТАЦІЇ: УРОКИ ДОСЛІДЖЕНЬ НА ТВАРИНАХ

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З нагоди 30-ї річниці створення Відділу ендокринології репродукції та адаптації Інституту ендокринології та обміну речовин ім. В.П. Комісаренка НАМН України в огляді розглядаються результати експериментальних досліджень у галузі формування нейроендокринної регуляції репродукції та адаптації в ранньому онтогенезі. Особлива увага приділяється статевій диференціації мозку та програмуванню розвитку системи гіпоталамус-гіпофіз-надниркові залози. Представлені перепрограмувальні перинатальні ефекти стероїдів, стресу, деяких ліків та хімічних ендокринних дисрапторів на мозок, що розвивається. Обговорюються феноменологія та нейрохімічні механізми, що лежать в основі гормон-нейромедіаторного імпринтингу морфології гіпоталамуса, статевої поведінки, репродуктивних та ендокринних функцій та стрес-реактивності. Результати досліджень можуть сприяти пренатальній профілактиці нейроендокринних та поведінкових розладів.

Ключові слова: мозок; статева диференціація; андрогени; естрогени; глюкокортикоїди; нейромедіатори; пренатальний стрес; статева поведінка; стрес-реактивність; ендокринні дисраптори; щури

А.Г. Резников

ФОРМИРОВАНИЕ НЕЙРОЭНДОКРИННОЙ РЕГУЛЯЦИИ РЕПРОДУКЦИИ И АДАПТАЦИИ: УРОКИ ИССЛЕДОВАНИЙ НА ЖИВОТНЫХ

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В связи с 30-летием создания Отдела эндокринологии репродукции и адаптации Института эндокринологии и обмена веществ им. В.П. Комиссаренко НАМН Украины в обзоре рассматриваются результаты экспериментальных исследований в области формирования нейроэндокринной регуляции репродукции и адаптации в раннем онтогенезе. Особое внимание уделяется половой дифференциации мозга и программированию развития системы гипоталамус-гипофиз-надпочечники. Представлены перепрограммирующие перинатальные эффекты стероидов, стресса, некоторых лекарств и химических эндокринных дисрапторов на развивающийся мозг. Обсуждаются феноменология и нейрохимические механизмы, лежащие в основе гормон-нейромедиаторного импринтинга морфологии гипоталамуса, полового поведения, репродуктивных и эндокринных функций и стресс-реактивности. Результаты исследований могут способствовать пренатальной профилактике нейроэндокринных и поведенческих расстройств.

Ключевые слова: мозг; половая дифференциация; андрогены; эстрогены; глюкокортикоиды, нейромедиаторы; пренатальный стресс; половое поведение; стресс-реактивность; эндокринные дисрапторы; крысы

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