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A.G. Reznikov, N.D. Nosenko, L.V. Tarasenko, P.V. Sinitsyn, A.A. Lymareva

Prenatal dexamethasone prevents early and long-lasting neuroendocrine and behavioral effects of maternal stress on male offspring

В роботі перевірено гіпотезу про роль гормонів гіпоталамо-гіпофізарно-адренокортикальної системи (ГГАС) в опосередкуванні індукованих материнським стресом нейроендокринних, нейрохімічних і поведінкових змін у нащадків чоловічої статі. Для гальмування стресової реакції ГГАС було використано дексаметазон, який вводили в дозі 0,1 мг/кг за 30 хв до початку одногодинної щоденної іммобілізації самицям щурів протягом останнього тижня вагітності. У пренатально стресованих самців раннього постнатального віку спостерігалося зникнення залежних від статі розбіжностей у розподілі білків та ароматазній активності в преоптичній ділянці гіпоталамуса. При досягненні статевозрілого віку у цих тварин виявлено послаблення копулятивної поведінки, зниження стресової та норадренергічної реактивності ГГАС. Введення дексаметазону в пренатальному періоді запобігало розвиткові зазначених вище змін, спричинених стресом материнського організму. Отримані результати свідчать про важливу роль гормонів ГГАС в опосередкуванні індукованих пренатальним стресом порушень процесів програмування розвитку мозку.

INTRODUCTION

A lot of early environmental influences on the developing mammalian brain like prenatal stress, exposure to excessive amount of glucocorticoids, sex steroids, nutrient restriction, maternal deprivation etc. were shown to be capable of modifying neuroendocrine functions and behavior in adulthood. Obviously, altered programming of the hypothalamic-pituitary-adrenal (HPA) axis that has resulted from maternal stress or glucocorticoid treatment in humans and animals might be associated with an impairment of HPA function, anxiety and depression-like behavior, high risk of hypertension, glucose intolerance, obesity and other metabolic disorders in adult offspring both in males and females [12, 26, 34]. Prenatal stress or glucocorticoid treatment of rats during the third week of pregnancy modifies the basic neurochemical and neurophysiological mechanisms of sexual brain differentiation and might contribute to sexual behavioral and reproductive disorders in adulthood [20, 22, 23, 29]. In human offspring, the adverse behavioral and cognitive consequences of stress experienced by the woman during natural environmental disaster have been described in ice-storm study [14].

Maternal stress during the last week of pregnancy generates numerous endocrine, neurotransmitter, cardiovascular and other homeostatic turbulences in the mother and fetus. Although HPA axis responses to stressors in late pregnancy seem to be attenuated [5], many studies focused at the possible key role of the HPA activation in the pathogenesis of long-term consequences of prenatal stress in primates, guinea pigs, rats, sheep, pigs and other species (for review see Kapoor et al., 2006). In rats, maternal stress leads to significant elevation of corticosterone levels in maternal and fetal plasma [10, 15, 31], and presumably to increased access of maternal

endogenous glucocorticoid to the fetus due to stress-induced reduction in placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2) activity (for review see Seckl et al., 2004). However, there are a lot of controversies with regard to mediating role of HPA hormones and mechanisms by which fetus exposure to stress *in utero* modifies the brain development programme.

It is still not clear whether stress-induced HPA activation generates multiple neuroendocrine and behavioral changes in prenatally stressed animals. Some prenatal stress effects, for example, changes in the hypothalamic noradrenaline content or sex-related HPA responses to noradrenergic stimulation in rats, could not be mimicked by fetal exposure to glucocorticoids [23, 30]. In the experiments with maternal bilateral adrenalectomy it was shown that maternal adrenal hormone mediates the HPA dysregulation and the alterations of anxiogenic and spatial learning behaviors in rat offspring experienced prenatal stress [36]. However, according to some research, prenatal ACTH or corticosterone treatments do not impair sexual behavior in male rats [10].

Bilateral adrenalectomy eliminates an increase in adrenal hormone release during stress. In the meantime, stressful stimuli provoke a wide range of homeostatic disturbances. Within HPA system, they involve surges of CRH, ACTH, vasopressin, β-endorphin secretion, hypothalamic noradrenaline depletion, glutamate excitation and many other events in neuroendocrine machinery.

In order to clarify the role of HPA function in pathogenesis of adverse neuroendocrine and behavioral consequences of prenatal stress in rats, dexamethasone (Dex) blockade of the HPA axis was used in this study followed by evaluation of the brain physiology and chemistry in prepubertal and mature male offspring.

EXPERIMENTAL PROCEDURES

Animals and experimental protocol

Experiments were performed according to protocols approved by the Animal Care Commission at the Institute, in accordance with European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986).

Virgin Wistar female rats (200±10 g) were mated overnight and pregnancy was confirmed by presence of spermatozoa in vaginal smears on following morning, which was considered gestational day 1. Time-mated dams (n=40) were subjected to 1 h strict immobilization in supine position during days 15 to 21 of gestation. Some of them (n=20) were injected i.m. with Dex (KRKA, Slovenia) in a dose of 0.1 mg/kg b.w. 30 min prior to each stress session. Control mothers (n=35) were injected with physiological saline according to the above protocol and housed in the vivarium with no handling.

Litter size averaged 6 pups. A total of 75 litters with 403 males were allocated into groups. 47 intact females were taken for comparison with control males. 5- and 10-day-old pups and mature descendants aged 3 months (weight range 140-160 g), 6 months (weight range 180 – 200 g) or 8 months (weight range 220 – 250 g) were taken to the study. The difference in the descendant body mass values within each animal group did not exceed 15 %.

Brain protein profile

Soluble cytosolic proteins isolated from the preoptic area (POA) and medial basal hypothalamus (MBH) were studied in 5-day-old offspring (n=140). The male pups from all experimental groups and both male and female control pups were quickly decapitated. POA and MBH were isolated by brain dissection, then combined by 5 - 7 tissue samples and frozen at –20 °C until being assayed. The spectrum of soluble proteins from discrete brain structures was determined by modified Laemmli method [16].

The tissues were homogenized in 10 volumes of ice-cold buffer, containing 0.25 M of sucrose, 0.025 M potassium chloride, 0.005 M magnesium chloride and 0.05 M tris-HCl,

pH 7.4, and centrifuged during 60 min at 100,000 g. Supernatant was taken for the elctrophoretic analysis. Protein concentration was measured by Lowry method [17]. Samples of soluble proteins containing 5 % SDS, 10 % mercaptoethanol and 40 % sucrose were heated at 100 °C for 5 min. Protein fractionation was carried out by plate disc-electrophoresis using 5 % polyacrylamide gel (PAAG) as concentrating and 10 % PAAG as dividing ones. Running buffer, pH 8.4, contained 0.025 M Tris-HCl, 0.1 % sodium dodecyl sulfate and 0.193 M glycine. Electrophoresis was carried out consecutively at 20 mA (in concentrating gel) and 30 mA (in dividing gel) for 40 – 60 min. Gels were stained overnight at room temperature in the mixture containing 9.0 % acetic acid, 45 % methanol and 0.025 % Coomassie Brilliant Blue R250, and then destained in several changes of 7.5 % acetic acid. LKB MW70 kit was used as molecular weight protein markers. Slab gels were scanned using Epson perfection 1670 scanner. Densitometry was carried out by Scion Image computer program. The results were expressed as relative density value under curve for each protein fraction with regard to total density.

Brain enzyme activities

Aromatase (estrogen sythetase, EC 1.14.14.1) and 5α-reductase (3-oxo-5a-steroid: $NADP^+$4-ene oxydoreductase, EC 1.3.1.22) activities were determined in POA and MBH of 10-day-old offspring (n=50). The male pups from all animal experimental groups and both male and female control pups were quickly decapitated. POA and MBH were isolated by brain dissection, then combined by 2 - 3 tissue samples and frozen at –20 °C until being assayed. For aromatase and 5α-reductase activities determination, the aliquots of 1000 g supernatant of the 10 % tissue homogenates were incubated during 1 h in Tris-HCl buffer (pH 7.4) containing [1,2,6,7-^{3}H]testosterone (s.a. 3.74 TBq/mmol, "Amersham", UK) as the enzyme substrate in the presence of NADP·H generating system (2 mM NADP, 10 mM glucose-6-phosphate and 2 IU/ml glucose-6-phosphate dehydrogenase) [18]. [4-^{14}C]Estradiol was used as an internal standard. Tritiated estradiol and 5α-reduced androgen metabolites which have been derived from testosterone were isolated by two-dimensional thin layer chromatography on Silica gel 60/Kieselguhr F_{254} plates ("Merck", Germany). Radioactivity of isolated steroids was recorded in a two-channel β-spectrometer (Beckman LS 500TA, USA). Aromatase activity was calculated as an amount of estradiol, and 5α-reductase activity as a sum of 5α-dihydrotestosterone and 3α-androstandiol produced for 1 h per 1 g tissue.

Sexual behavior evaluation

Male sexual behavior tests were run in two trials. The male rats (n=19) were 87-90 days old in the first trial and 93-96 days old in the second one. The control, prenatally stressed and Dex-pretreated prenatally stressed males, were tested during the second half of the dark cycle with ovariectomized female rats made receptive by hormonal injection (0.1 mg of estradiol benzoate per rat, i.m. in oil, 48 h prior to the test day followed by 0.5 mg of progesterone in oil i.m., 4 h prior to the test). The test consisted of placing each male to mating chamber 5 min before a receptive female was introduced. Mating chambers were illuminated with dim red lights. Each test lasted for 30 min. The frequency of mounts, intromissions and ejaculations, and the timing of each response as well as time to first mount after the first ejaculation (postejaculatory interval) were recorded. This procedure was repeated in one week. Results were assessed separately for the first and the second week.

Stress-test procedure

6-Month-old male offspring (groups of control, prenatally stressed and Dex-pretreated prenatally stressed males, each consisted of 5-6 animals) was subjected to 1 h strict restraining. The rats were immobilized in supine

position with the extremities being attached to a small animal desk. Non-stressed animals were used as controls. The animals were quickly decapitated immediately after 1 h restriction. Taking into consideration possible impact of general anaesthesia on the catecholamine content in the brain tissues euthanasia was performed without anaesthetic drugs. The hypothalamus was isolated by brain tissue dissection at +4 °C. Only fresh brain tissues were used for catecholamine assay. Trunk heparinized blood samples were taken, and the plasma was separated and stored at –20 °C until measurement of corticosterone concentrations.

Study of baclofen effects on the HPA stress responses

The experiments were carried out in 6-month-old male offspring (n=48). Baclofen, $GABA_B$-receptors agonist ("Sigma", USA), dissolved in apyrogenic isotonic NaCl solution was injected i.p. in a dose of 10 mg/kg b.w. 30 min prior to an acute stress (1 h restriction). The animals were decapitated immediately after 1 h restriction. Trunk heparinized blood samples were taken, and the plasma was separated and stored at -20 °C until measurement of corticosterone concentrations.

Study of the HPA responses to noradrenergic stimuli

The experiments were carried out in 8-month-old male offspring (groups of control, prenatally stressed and Dex-pretreated prenatally stressed males, each consisted of 6 animals). Noradrenergic reactivity of the HPA axis was tested in conscious unrestrained animals. Noradrenaline bitartrate in a dose of 10 μg dissolved in 2 μl of apyrogenic isotonic NaCl solution (saline) was infused into the 3rd brain ventricle via a needle inserted into a stainless steel guide cannula that had been implanted 8 to 9 days before the experiment using stereotaxic coordinates [2]. Twenty four hours prior to the experiment, a Silastic catheter was inserted into the right external jugular vein [9]. Surgery was performed under ether anesthesia. Blood samples (0.5 ml) were taken from jugular vein before and then 30 and 60 min after noradrenaline infusion. Every sample was substituted immediately by an equal volume of saline containing 50 IU/ml heparin. Plasma was separated and the aliquots were stored at -20 °C until measurement of corticosterone concentrations. As it was revealed in the previous studies, intracerebroventricular infusions of 2 μl saline do not affect plasma corticosterone levels.

Catecholamine assay

Noradrenaline and dopamine (3-hydroxytyramine) contents were determined in the hypothalamus of 6-month-old male offspring. Immediately after decapitation, hypothalamus was excised as rapidly as possible, then weighed and frozen on dry ice. Hypothalamic tissue samples were homogenized on ice in 0.01 N HCl (1:50 w/v). Catecholamines were extracted with 5 ml of n-butanol. After centrifugation at 1500 g for 10 min, the supernatants were placed to the tubes containing pH 6.5 phosphate buffer in order to transfer catecholamines to aqueous phase. All of these procedures were performed at 4 °C. Noradrenaline and dopamine contents were determined by spectrofluorimetric assay [11] in 0.5 ml aliquotes of supernatant after centrifugation at 3000 g. Catecholamine fluorophores were produced by oxidation of noradrenaline and dopamine by adding 125 μl of Versen solution, 100 μl 0.3 M KI and 125 μl 0.5 M Na_2SO_3 in 2 min intervals. The reaction was stopped by adding 150 μl 5 N acetic acid. The test tubes were placed into boiling water for 5 min followed by 1 min cooling in ice-bath. Fluorescence of noradrenaline and dopamine was measured immediately using 385/485 nm and 320/385 nm optical filters, correspondingly. Noradrenaline bitartrate and 3-hydroxytyramine hydrochloride ("Sigma", USA) were used as standards. The concentrations of the monoamines were expressed as nmol/g of tissue.

Plasma corticosterone assay

Blood plasma corticosterone concentrations were measured by spectrofluorimetric microassay [3]. 100 μl blood plasma samples

were extracted with 1.5 ml methylene chloride for 3 min. Aqueous phase containing cholesterol and other lipids was frozen at –40 °C. Organic extracts were poured into the test tubes, and 0.5 ml of fluorescent reagent containing research grade H_2SO_4 and dehydrated ethanol (7 : 3, v/v) was added. The content of the tubes was mixed and the upper methylene chloride layer was discarded. Corticosterone fluorescence was measured in 1.5 h using 470/524 nm optical filters.

Statistical analysis

All data are presented as mean ± SEM. Student's *t* criterion or Wilcoxon's *U* test were used for evaluation of the differences between experimental groups. $P < 0.05$ was considered as the borderline of statistic significance.

RESULTS

Brain protein profile

In the POA of 5-day-old normal control pups, a significant sexual dimorphism in the density of soluble proteins with molecular weights of 14.3 – 66.0 kDa was observed. The 14.3 kDa and 24.0 kDa molecular weight proteins were denser in females (respectively, 10 % and 15 %, $p<0.05$) while the proteins with 34.7 and 66.0 kDa molecular weight – in males (respectively, 30 % and 10 %, $P<0.05$). There were no gender-related differences in the electrophoretic profiles of proteins studied in the MBH.

As a result of maternal stress, diminution of the density of 66.0 kDa protein was found in the POA of males (Fig. 1). This modifying effect of prenatal stress on the protein was prevented by Dex. Prenatal stress alone or combined with Dex pretreatment did not change the densities of any soluble proteins in the MBH of male pups.

Brain enzyme activities

In the POA of prenatally stressed 10-day-old pups, aromatase activity declined on average by 41.1 % (Fig. 2) and reached the normal female level (for comparison, 0.352 ± 0.072 nmol estradiol·h^{-1}·g tissue^{-1} in females *vs.* 0.598 ± 0.082 nmol estradiol·h^{-1}·g tissue^{-1} in males, $P<0.05$). Dex pretreatment completely restored the enzyme activity to normal level in prenatally stressed males.

Prenatal stress did not affect aromatase activity in the MBH tissue samples in males,

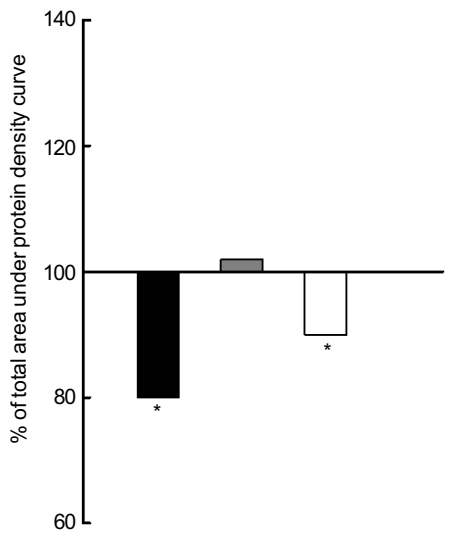

Figure 1. Changes in 66.0 kDa protein profile in the brain preoptic area (POA) in prenatally stressed (dark bar), Dex-pretreated prenatally stressed (gray bar) 5-day-old males and control 5-day-old females (light bar) related to control 5-day-old males (accepted as 100 %). *P<0.05 compared to control 5-day-old males (Wilcoxon's *U*-test)

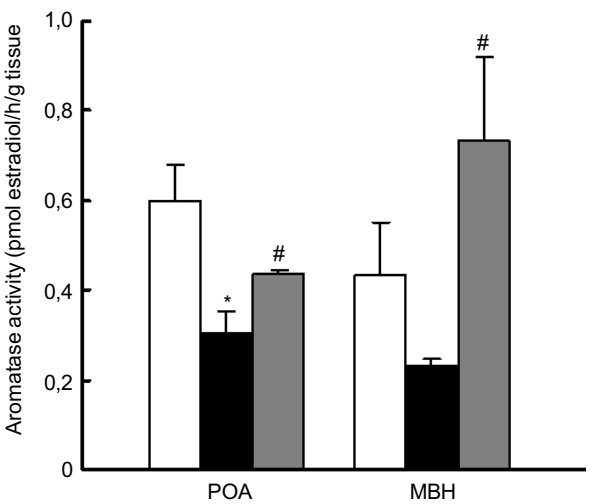

Figure 2. Aromatase activity in the brain preoptic area (POA) and medial basal hypothalamus (MBH) in control (light bars), prenatally stressed (dark bars) and Dex-pretreated prenatally stressed (gray bars) 10-day-old males. Data are means ± SEM. *P<0.05 compared to control group; #P<0.05 compared to prenatally stressed males (Student's *t*-test)

Table 1. Effects of prenatal exposure to Dex on brain 5α-reductase activity in prenatally stressed 10-day-old male rats

Groups	5α-reductase activity, pmol 5α-reduced metabolites·h^{-1}·g tissue^{-1}	
	POA	MBH
Control (n=6)	5.80 ± 0.83	5.87 ± 0.78
Prenatally stressed (n=5)	8.53 ± 2.86	14.51 ± 3.40*
Dex-pretreated prenatally stressed (n=6)	8.46 ± 1.14	8.95 ± 1.60

* P<0.05 compared to controls

while in Dex-pretreated ones significant increase of enzyme activity compared to prenatally stressed males without Dex premedication was observed.

The data of measuring 5α-reductase activities are presented in Table 1. In the POA of control males it was twice lower, and in the MBH it was almost 3.5 times less than in female pups (POA: 11.61 ± 2.06 pmol 5α-reduced metabolites·h^{-1}·g tissue^{-1}, P<0.05; MBH: 20.37 ± 2.30 pmol 5α-reduced metabolites·h^{-1}·g tissue^{-1}, P<0.001). Prenatal stress resulted in significant rise of testosterone reduction in the male MBH up to values which did not statistically differ from those in normal females, while there were no changes in the POA. As for 5α-reductase activity, Dex-pretreated prenatally stressed males did not significantly differ either from control or from prenatally stressed pups.

Sexual behavior

When male offsprings were tested for sexual performance in adulthood, some variables of male sexual behavior improved over 2 weeks of testing due to sexual experience acquisition and development of stereotype reactions. Therefore, the results are presented separately for the first and the second week (Table 2). Actually, latencies to first mount and ejaculation in all animal groups, as well as ejaculation frequency, in contol males significantly improved in time course. Prenatal stress re-

Table 2. Effects of prenatal exposure to Dex on male sexual behavior in prenatally stressed 3-month-old male rats

Variable	Groups		
	Control (n = 6)	Prenatally stressed (n = 6)	Dex-pretreated prenatally stressed (n = 7)
	1st trial		
Latency (sec):			
to first mount	10.8 ± 2.9	33.6 ± 7.8*	28.2 ± 8.2
to first intromission	15.8 ± 4.3	38.1 ± 10.6	32.2 ± 9.8
to first ejaculation	617.5 ± 87.1	727.8 ± 44.3	657.2 ± 59.2
Postejaculatory interval (sec)	266.3 ± 79.9	665.7 ± 86.5**	225.3 ± 30.9
Number of ejaculations	2.2 ± 0.2	1.1 ± 0.2**	2.0 ± 0.3^
	2nd trial		
Latency (sec):			
to first mount	3.5 ± 0.5#	12.3 ± 0.9***,#	5.8 ± 1.9#
to first intromission	4.0 ± 0.6	25.0 ± 12.0*	9.5 ± 4.1
to first ejaculation	368.8 ± 56.7#	470.8 ± 81.4#	421.5 ± 57.1#
Postejaculatory interval (sec)	128.8 ± 44.5	260.8±27.2**,###	188.3 ± 47.0
Number of ejaculations	3.5 ± 0.3##	1.5 ± 0.2***	2.3 ± 0.2**,^

* P<0.05, ** P<0.01, *** P<0.001 compared to controls; # P<0.05, ## P<0.01 compared to the 1st trial; ^ P<0.05 compared to the prenatally stressed males

sulted in significant prolongation of the first mount latency period and decrease in ejaculation frequency both in the 1st and the 2nd trials. Furthermore, in the 2nd trial the time prior to the first intromission was significantly longer than that of control males. Prenatal exposure to Dex has led to male sexual behavior normalization in the 2nd trial on the whole. The only exception was the number of ejaculations during the 2nd mating session that was still significantly fewer versus control indices but greater compared to that of prenatally stressed rats.

HPA responses to an acute stress

Prenatally stressed mature males exhibited decreased corticosterone responses to 1 h restraint with no changes in basal corticosterone levels (Fig. 3). Basal dopamine concentration in the hypothalamus was 15.7 % higher than those of normal control ($P<0.05$), while noradrenaline maintained at normal levels. HPA stress-activation was not followed by a decrease in hypothalamic noradrenaline content, although control males exhibited its depletion (24.2 %, $P<0.001$) (Table 3, *experiment 1*).

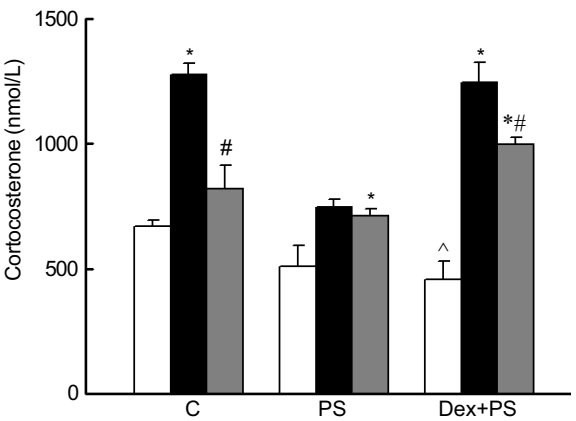

Figure 3. Effects of baclofen pretreatment on blood plasma corticosterone response to 1h restriction in control (C), prenatally stressed (PS) and Dex-pretreated prenatally stressed (Dex + PS) 6-month-old male offspring. Values are means ± SEM of 5-6 animals per group. Light bars - basal levels; dark bars - after 1 h restriction; gray bars - after 1 h restriction with baclofen pretreatment. *P<0.01 compared to basal level; # P<0.05 compared to level after 1 h restriction; ^ P<0.05 compared to resting level in control group (Student's *t*-test).

Prenatal Dex treatment rendered protective effect with regard to diminishing adrenocortical and hypothalamic noradrenaline responses to an acute stress in those animals. Dex-pretreated prenatally stressed males showed decreased basal plasma corticosterone level (31,6 %, $P<0.05$) but corticosterone response to an acute stress was similar to that of control rats (Fig. 3). There were no significant differences in stress-induced declines of hypothalamic noradrenaline contents between the control (24.2 %, $P<0.01$) and Dex-pretreated prenatally stressed males (20 %, $P<0.05$) (Table 3, *experiment* 2). As well no changes were found in resting hypothalamic catecholamine contents in the Dex-pretreated prenatally stressed males compared to normal control.

Baclofen effects on the HPA stress responses

Pretreatment with baclofen diminished stress-stimulated secretion of corticosterone in control males but failed to alter this parameter in prenatally stressed rats (Fig. 3). Dex-pretreated prenatally stressed males, as well as control ones, showed significant reduction in the adrenocortical response to an acute stress following treatment with baclofen but the amplitude of blood plasma corticosterone level drop was less than that of control.

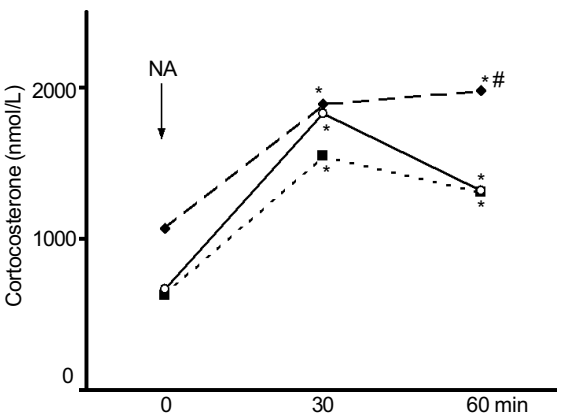

Figure 4. HPA response to intracerebroventricular noradrenaline (NA) infusion in control (continuous line), prenatally stressed (dotted line) and Dex-pretreated prenatally stressed (pointed line) 8-month-old males. Data are means ± SEM. *P<0.05 compared to basal level, #P<0.05 compared to control group (Student's *t*-test).

Table 3. Hypothalamic catecholamine response to 1 h restriction in control, prenatally stressed and Dex-pretreated prenatally stressed 6-month-old males

Groups	Catecholamine (nmol/g tissue)	
	Noradrenaline	Dopamine
Experiment 1		
Control:		
Resting level	8.36 ± 0.36	4.64 ± 0.20
After 1 h restriction	6.34 ± 0.17 **	4.82 ± 0.15
Prenatally stressed:		
Resting level	8.00 ± 0.17	5.37± 0.21#
After 1 h restriction	7.21 ± 0.59	5.29 ± 0.49
Experiment 2		
Control:		
Resting level	7.15 ± 0.31	4.83 ± 0.38
After 1 h restriction	5.42 ± 0.39 ***	4.40 ± 0.37
Dexamethasone-pretreated prenatally stressed:		
Resting level	6.75 ± 0.46	4.67 ± 0.30
After 1 h restriction	5.40 ± 0.34 *	4.96 ± 0.12

Data are means ± SEM of 5-6 animals per group. *P<0.05; ** P<0.01; ***P<0.001 compared to resting levels; # P<0.05 compared to resting level in control group (Student's *t* test).

HPA responses to noradrenergic stimuation

Noradrenaline infusion into the 3rd brain ventricle resulted in almost 3-fold blood plasma corticosterone rise in control males at the 30th min (Fig. 4). In the sequel, it lowered by the 60th min but was still significantly higher as compared to basal level. Prenatal stress evoked hyperactivation of the HPA axis following noradrenaline application in male offspring: blood plasma corticosterone continued to elevate by the 60th min. Though the extent of response in those animals was less pronounced than in controls (rise above basal level by 77 and 175 %, respectively, P<0.05).

The pattern of adrenocortical response to noradrenaline central stimulation in Dex-pretreated prenatally stressed males was similar to normal one with the only exception of reaction amplitude, which was somewhat lower at the 30th min in comparison with control male rats (rise by 147 vs. 175 % in controls, P>0.1).

DISCUSSION

Dex, synthetic glucocorticoid, administered in this study in a dose of 0.1 mg/kg b.w, is commonly used for suppressing the HPA axis in the rat [1]. High glucocorticoid activity of Dex is connected with its high affinity to tissue glucocorticoid receptors, as well as low binding to plasma proteins [7]. Due to binding to hippocampal glucocorticoid and, to less extent, to mineralocorticoid receptors [12], which mediate corticosteroid feedback control of basal and stress-activated adrenal hormone secretion, Dex attenuates the HPA axis response to stress.

In this study Dex was used for suppression of stress-stimulated HPA hormone secretion in order to examine whether the hormones of the HPA axis mediate early and long-term neurochemical, neuroendocrine and behavioral effects of prenatal stress in male rats. Dex is a poor substrate for 11β-HSD-2, which prevents the fetus from excess of maternal glucocorticoids, and therefore it readily passes the placenta [29]. For this reason it should attenuate stress-stimulated HPA function both in pregnant mother and the fetus.

Here it has been shown for the first time that supression of HPA hormone secretion with Dex during restraining rats in late gestation prevents numerous early and long-term

alterations including brain tissue protein pattern, brain testosterone metabolism, male sexual behavior, hippocampal $GABA_B$ receptor, hypothalamic noradrenaline and plasma corticosterone responses to an acute stressor or noradrenergic stimulation of HPA function.

Our findings on maintenance of normal hypothalamic noradrenaline and adrenocortical responses to an acute stress in adult rats exposed *in utero* to stress with Dex pretreatment support the concept on the mediating role of adrenal glucocorticoid excess in modulating effect of prenatal stress on developmental programming of HPA hormone activity [12, 22, 35].

Previously we revealed that adult male rats exposed prenatally to exogenous glucocorticoid demonstrate a diminution in plasma corticosterone response to noradrenaline infused into the 3rd brain ventricle [30], meanwhile, in prenatally stressed males a hypersensitivity of the HPA axis to noradrenergic stimulation was found [25]. This unexpected effect of prenatal glucocorticoid treatment could appear for its direct influence on developing neurotransmitter systems, in particular on tyrosine hydroxylase gene expression [6]. Given preservation of noradrenergic sensitivity of the hypothalamus, the main trigger of the HPA axis, in Dex-pretreated and prenatally stressed male offspring, we hypothesize that neurotransmitter disturbances in the stressed fetal brain could contribute to alteration of programming of noradrenergic control of HPA function resulted from prenatal stress.

Neuroendocrine control of HPA hormone secretion under stress condition involves GABA-ergic limitation mechanism mediated by $GABA_A$ and $GABA_B$ receptors. This mechanism tested with baclofen, $GABA_B$ receptor agonist, was found to be impaired in male rats following prenatal stress. Prenatal exposure of males to exogenous cortisol (hydrocortisone acetate) exerts similar effect [19]. On the contrary, decreased adrenocortical responses to an acute stress after pretreatment with baclofen were observed both in normal and Dex-pretreated prenatally stressed male rats, which suggests the ability of $GABA_B$ receptor to additional activation. Taken together, these findings reflect mediating role of stimulated HPA hormone secretion in maternal stress-induced alteration of $GABA_B$ receptor function in male offspring.

Maternal stress during the last week of gestation leads to demasculinization and/or feminization of sexual behavior in adult male offspring resulted from disorder of androgen-dependent sexual brain differentiation. This generally recognized phenomenon (prenatal stress syndrome) has been partially reproduced in this study by appearance of insufficient male copulatory behavior.

In male rodents, the POA is known to play a key role in neuroendocrine regulation of male sexual behavior. Decrease in male sex-associated 66.0 kDa protein density in the POA of prenatally stressed 5-day-old animals can be considered as an early sign of impaired sexual brain differentiation, though we cannot identify its function. As far as steroid aromatase activity in the POA of 10-day-old pups is concerned, it must be taken into account, that this enzyme is one of the main determinants of androgen-dependent brain differentiation [18, 23] and it plays crucial role in maintaining all aspects of sexual behavior in male rats [28]. Therefore, decrease in the enzyme activity resulted from prenatal stress reflects the process of demasculinization of male sexual behavior, which was confirmed by its testing in adult offsprings.

We have not investigated the lordosis reaction in males in this study. Given location of neuroendocrine regulation of female sexual behavior which in rodents is associated with the MBH, one can suggest that 5β-reductase activity elevation in this brain structure in prepubertal prenatally stressed males might result not only in demasculinization but presumably in feminization of their sexual behavior in adulthood.

Since steroid aromatase in the POA of male rats is induced by testosterone [27], the decline of the enzyme activity in the critical period of sexual brain differentiation presumably results from androgen insufficiency in the fetus under stress condition. It is generally believed that prenatal stress syndrome is generated by an increase of maternal adrenal glucocorticoids in blood circulation with concomitant transitory testicular androgen deficit in male fetuses. Our recent manipulations with testosterone replacement or naltrexone, opioid receptor antagonist, treatment of stressed pregnant rats have demonstrated the essential role of androgen shortage and endogenic opioids in early neurochemical feminization of the brain in prenatally stressed male rats [21, 24] which is in line with the results of studying of adult male sexual behavior in similar experiments [4, 13, 33].

Catecholamines (both noradrenaline and dopamine) are known to play primarily stimulatory or permissive role in sexual behavioral reactions [32]. Our findings on dopamine increase in the MBH of prenatally stressed adult male rats are quite matched with the disorders in male sexual behavior. Moreover, these observations are in concordance with the results reported by Gerardin et al. [8]. Dex pretreatment of prenatally stressed males was shown to restore both normal dopamine level in the MBH and consequently normal manifestation of motivational and copulatory components of male sexual behavior.

The results of this study suggest that prenatal Dex given prior to restraint of pregnant dams interrupted the chain of physiological events in maternal and fetal bodies associated with an impairment of sexual brain differentiation. In adult males such Dex premedication leads to almost complete normalization both of sexual appetite and sexual performance. Taken together with Dex preventive effects on HPA function in prenatally stressed male rats, these results support the conclusion that HPA axis hormone secretion with opioids, adrenal glucocorticoids, and, probably, other messengers of stress response included mediates alterations of programming of brain development induced by prenatal stress.

A.G. Reznikov, N.D. Nosenko, L.V. Tarasenko, P.V. Sinitsyn, A.A. Lymareva

PRENATAL DEXAMETHASONE PREVENTS EARLY AND LONG-LASTING NEUROENDOCRINE AND BEHAVIORAL EFFECTS OF MATERNAL STRESS ON MALE OFFSPRING

The hypothesis on the mediating role of hypothalamic-pituitary-adrenocortical (HPA) hormone secretion in neuroendocrine, neurochemical and behavioral alterations generated by prenatal stress in male rat offspring was tested in this study with dexamethasone (Dex) used for suppression of HPA stress responses. Pregnant dams were being restrained daily for 1 h over the last week of gestation. In male offspring this resulted in attenuation of sex-specific pattern of the protein fractions (on the 5th postnatal day), steroid aromatase activity (on the 10th postnatal day) in the brain preoptic area, and in a decrease of male copulatory behavior, hypothalamic noradrenaline and plasma corticosterone responses to an acute stress, an increase in HPA responses to noradrenergic stimulation and other effects in adulthood. All those changes were prevented with prenatal Dex in a dose of 0.1 mg/kg b.w. injected 30 min prior to restraining pregnant dams. As such, HPA hormone secretion mediates alterations of programming of brain development induced by prenatal stress.

V.P.Komisarenko Institute of Endocrinology and Metabolism, Kyiv

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V.P.Komisarenko Institute of Endocrinology and Metabolism, Kyiv
E-mail: dccie@mail.kar.net

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