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Catecholamines in Steroid-dependent Brain Development

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Sex-specific peculiarities of catecholamine (CA) content and turnover in neuroendocrine brain areas and their modification with neonatal steroids or prenatal stress (PS) in Wistar rats were studied. No changes in noradrenaline (NA) content and turnover rate were found in the preoptic area (POA), meanwhile dopamine (DA) turnover rates in the POA and mediobasal hypothalamus (MBH) were increased in neonatally androgenized 10-day-old females. Treatment of female neonates with various catecholestrogens increased hypothalamic NA content by 30–95% but only 4-hydroxy-estradiol-17β induced anovulation. 6-Hydroxydopamine had no significant impact on hypothalamic CA content in neonates and did not prevent testosterone-induced persistent estrous. Maternal stress (restriction for 1 h a day, 15–21st days of pregnancy) resulted in a decrease of hypothalamic NA and blood plasma corticosterone response to acute stress in adult male offspring. Sex differences in CA content in the POA and MBH disappeared in 10-day-old prenatally stressed rats. Conclusions: (1) sexual brain differentiation needs co-operative actions of sex steroids and CA to be completed; and (2) early changes in CA content and turnover induced by PS or neonatal steroid exposure predetermine long-term alterations of the stress responsiveness, reproductive behaviour and neuroendocrine control of ovulation.

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INTRODUCTION

In early ontogenesis gonadal and adrenocortical steroids play the role of organizers of the developing brain and determine a number of essential features of neuroendocrine regulation of behaviour [1, 2], stress-reactivity [3, 4] and reproduction [5–7]. Numerous experimental data show that catecholamines (CA) and, perhaps, other neurotransmitters as well are involved in sexual brain differentiation (SBD) [8–11].

Pharmacologic analysis with blockers of synthesis or metabolism of biogenic monoamines, steroid aromatase inhibitors, catecholestrogen (CE) administration demonstrated the important role of androgen and CA metabolism and their interrelation at the critical period of SBD [12–15]. However, neurochemical mechanisms of hormone-neurotransmitter imprinting of the developing brain need further investigation. The role of CE isomers, as possible inducers of SBD and modulators of brain CA metabolism, remains obscure. In particular, it seems reasonable to study CA turnover in discrete brain regions responsible for neuroendocrine control of sexual behaviour and ovulation in the disturbance of steroid hormone equilibrium in early ontogenesis.

Disorders of stress-induced reactivity and sexual behaviour in adult male rat offspring as a result of maternal stress during pregnancy may be caused by endogenous hypercorticoidism, decreased LH and T levels, and neurotransmitter imbalance in mothers and fetuses [16–19].

In this study sex-specific peculiarities of CA content and turnover in the preoptic area (POA) and mediobasal hypothalamus (MBH) of rats caused by administration of testosterone propionate (TP), CE isomers and 6-hydroxydopamine (6-OHDA) during early postnatal life were investigated. The effects of maternal stress on sexual differences in CA content in the POA and MBH in pups, and hormonal and CA responsiveness to acute stress in adult male offspring were studied.

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MATERIALS AND METHODS

Chemicals

α-Methyl-*p*-tyrosine methyl ester (MPT, Koch-Light), 6-OHDA hydrochloride (Serva), 2-hydroxyestrone (2-OHE_1) and 2-hydroxyestradiol-17β (2-OHE_2-17β) (Steraloids). 4-Hydroxyestradiol-17β (4-OHE_2-17β) and 4-hydroxyestradiol-17α (4-OHE_2-17α) were kindly provided by Dr Knuppen (Germany).

Animals and experimental design

Experiments were carried out in neonate and adult Wistar rats (date of birth was considered the first day of life). Animals were maintained in a controlled environment. Pups were treated with TP in oil solution (50 μg per rat s.c. on postnatal day 5), 6-OHDA dissolved in saline with 0.1% ascorbic acid (100 mg/kg b.w. s.c. on postnatal days 1 and 2) or CE isomers (10 μg per rat s.c. daily on postnatal days 1–5). Control animals were given solvents. Prenatally stressed rats were obtained from mothers which were exposed to 1 h strict immobilization daily on the 15–21st days of pregnancy. Animals were decapitated on the 5th or 10th day after birth.

When CA turnover rate was studied in 10-day-old pups, MPT, a tyrosine hydroxylase inhibitor, dissolved in saline (250 mg free base/kg b.w.) was injected s.c. 1 or 2 h prior to decapitation. The brain was removed immediately and the hypothalamus or POA and MBH were isolated at +4°C and used for CA assay.

In 3-month-old females neonatally treated with 6-OHDA, vaginal smears were examined daily for 2 weeks. Adrenocortical response to acute stress (1 h immobilization) was tested in 3-month-old prenatally stressed males. The animals were decapitated immediately after immobilization. The hypothalamus was isolated and subjected to CA assay. Heparinized plasma samples were prepared for corticosterone measurements.

CA assays and calculation of CA turnover rates

The hypothalamus, POA and MBH tissue samples taken from 2–3 pups were combined and homogenized in 0.01 N HCl (1:50 w/v) and centrifuged at 1500 *g* for 10 min at +4°C. 0.5 ml aliquots of the supernatant were used to estimate noradrenaline (NA) and dopamine (DA) content by spectrofluorimetric assay [20]. CA turnover rates were calculated according to Brodie *et al.* [21].

Corticosterone assay

Blood plasma corticosterone levels were measured by fluorimetric method [22].

Statistical analysis

Student's *t*-test or Wilcoxon's U-test were used to determine the significance of differences ($P < 0.05$) between mean values for control and tested groups.

RESULTS

Sexual differences in neonatal brain CA content and turnover

NA content in the POA of 10-day-old female rats was higher by 48.8% when compared to males, while no difference in DA content was observed (Table 1).

Table 1. Sexual differences in neonatal brain CA content and the effects of maternal stress

Animal group	POA		MBH	
	NA	DA	NA	DA
Females				
Intact	4.39 ± 0.56	3.98 ± 0.19	1.95 ± 0.15	2.43 ± 0.14
Prenatally stressed	3.49 ± 0.44	3.05 ± 0.26*	1.73 ± 0.04	3.26 ± 0.29*
Males				
Intact	2.95 ± 0.18*	3.85 ± 0.36	2.56 ± 0.27‡	4.72 ± 0.75*
Prenatally stressed	4.71 ± 0.64†	5.94 ± 1.57	2.07 ± 0.14‡	3.84 ± 0.49*

Data represent means ± SEM (nmol/g tissue) of 5–6 determinations. *$P < 0.05$ vs intact females; †$P < 0.05$ vs intact males; ‡$P < 0.05$ vs prenatally stressed females.

On the contrary, in female MBH, DA concentration was found to be less by 48.5% than in males. In this experiment no statistically significant sexual differences were found regarding NA concentration in the MBH. In another experiment, NA concentrations in male and female MBH were different making 3.39 ± 0.26 and 2.56 ± 0.16 nmol/g tissue, respectively ($P < 0.05$).

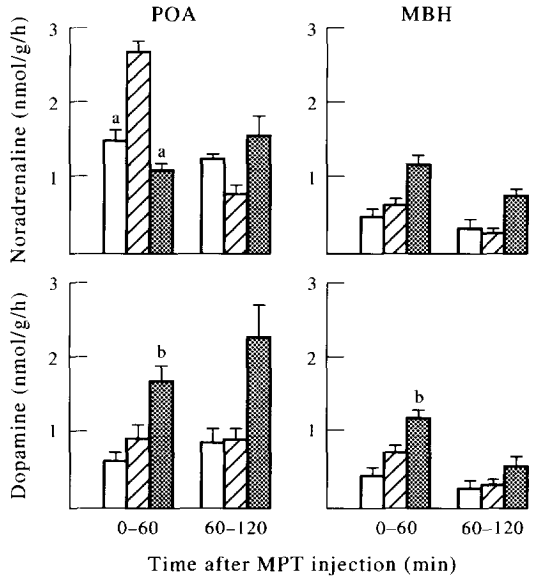

Fig. 1. Catecholamine turnover rates in the brain of normal and androgenized rats on the 10th postnatal day. Each bar represents mean ± SEM of 5 determinations. □, normal females; ▨, normal males; ▩, androgenized females. [a]$P < 0.05$ vs normal males; [b]$P < 0.05$ vs normal females.

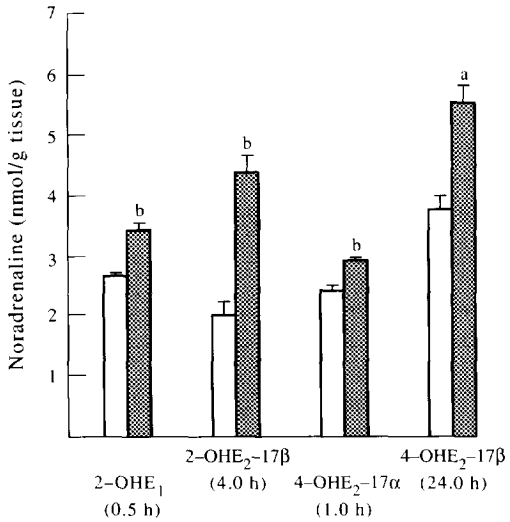

Fig. 2. Effects of catecholestrogens (10 μg daily on the 1st–5th postnatal days) on noradrenaline concentration in the female rat hypothalamus. Each bar represents the mean ± SEM of 5–11 determinations. □, control; ▨, catecholestrogen injection. Times of maximum increase of noradrenaline concentrations are presented in parentheses. $^aP < 0.05$; $^bP < 0.02$ vs control.

In line with lower NA content in the POA its turnover rate in males during 1 h after MPT injection was 1.8 times that in females (Fig. 1). There were no significant male–female differences in CA turnover rates in the MBH and DA in the POA for 1 h after MPT administration, as well as in CA turnover during the blockade of tyrosine hydroxylase in the subsequent hour.

Effects of neonatal androgenization on CA content and turnover

Sexual differences in NA content and turnover in the POA of 10-day-old rats were not affected by TP treatment of female neonates, while DA turnover in the POA and MBH was accelerated (Fig. 1). NA and DA content in the MBH of neonatally androgenized female pups was higher by 35.9 and 67.4%, respectively, as compared with intact females ($P < 0.05$). At the same time NA turnover in this area in androgenized females exceeded that of intact females 2.4 times with the level of statistical significance being approximated to $P < 0.05$.

Effects of neonatal administration of CE isomers to females

The time course of hypothalamic NA concentration changes in neonates were different depending upon the CE isomers administered. All isomers elevated NA concentration in the hypothalamus. Terms of maximum NA increase and its values are shown in Fig. 2. Only 2-OHE_2-17β provoked an increase in hypothalamic DA concentration. In contrast to 2-OHE_1, 2-OHE_2-17β and 4-OHE_2-17α, only 4-OHE_2-17β induced persistent estrous in 80% of adult rats.

Table 2. CA concentration in the hypothalamus of 10-day-old female rats treated with TP and/or 6-OHDA for the first 2 days after birth

Treatment	NA	DA
Solvent	3.34 ± 0.33	2.82 ± 0.49
TP	4.54 ± 0.38*	4.72 ± 0.27†
Solvent	3.32 ± 0.19	6.37 ± 0.46
6-OHDA	2.84 ± 0.12	4.99 ± 0.52
TP	3.74 ± 1.11	5.64 ± 0.37
TP + 6-OHDA	2.45 ± 0.09	5.74 ± 0.54

Data represent means ± SEM (nmol/g tissue) of 4–5 determinations. *$P < 0.05$; †$P < 0.001$ vs solvent.

Neonatal administration of 6-OHDA to females

6-OHDA administered to female neonates either alone or in combination with TP had no significant impact on hypothalamic NA and DA concentrations 10 days after birth (Table 2).

Under separate administration, 6-OHDA did not alter the estrous cycle in rats aged 3 months. When combined with TP, it did not prevent TP-induced persistent estrous.

Short- and long-term effects of maternal stress

Maternal stress prevented sexual dimorphism in concentrations of NA in the POA and DA in the MBH on the 10th day of postnatal life (Table 1). NA content in the POA in male offspring rose up to the level of that in intact females, while DA content in the MBH of female offspring increased up to the normal male level. NA content in the MBH of male and female offspring was not affected by maternal stress.

As a result of maternal stress diminution of corticosterone response to acute immobilization stress was found in adult males: whereas basal corticosterone levels in intact and prenatally stressed rats were similar (0.76 ± 0.01 vs 0.72 ± 0.02 mmol/l), following immobilization corticosterone concentration increased by 162 and 54%, respectively ($P < 0.001$). Acute stress caused the lowering of hypothalamic NA concentration

Table 3. Hypothalamic CA and blood plasma corticosterone response to acute stress in intact and prenatally stressed adult rats

Animal group	NA (nmol/g tissue)	DA (nmol/g tissue)	Corticosterone (mmol/l)
Intact			
Control	8.36 ± 0.36	4.64 ± 0.20	0.76 ± 0.01
Immobilization	6.34 ± 0.17*	4.82 ± 0.15	1.99 ± 0.03*
Maternal stress			
Control	8.00 ± 0.17	5.37 ± 0.21	0.72 ± 0.02
Immobilization	7.21 ± 0.59	5.29 ± 0.49	1.11 ± 0.03†‡

Data represent means ± SEM of 5–6 determinations. *$P < 0.05$; †$P < 0.001$ vs respective control; ‡$P < 0.01$ vs immobilization of intact rats.

in intact males from 8.36 ± 0.36 to 6.34 ± 0.17 nmol/g tissue ($P < 0.001$), such a response was not observed in prenatally stressed animals. DA content was tolerant to acute stress in all experimental groups.

DISCUSSION

The findings in this study give new evidence in favour of the concept on the dependence of CA in the neuroendocrine brain upon androgen influence during early postnatal life [12,23]. Sexual dimorphism of CA content and turnover in the POA and MBH is of a different character, evidently, it is related to the different functions these brain areas perform in neuroendocrine control of reproduction.

4-OHE_2-17β, produced in nervous tissue as a result of androgen metabolism, is a probable mediator of testosterone masculinizing effects on the developing female brain. In contrast to this catecholestrogen, its 17α-isomer as well as 2-hydroxylated estrogens, administered to neonates, did not produce anovulatory syndrome despite an elevation of hypothalamic NA content. This might be explained by their weak estrogenic activity due to low affinity to cellular estrogen receptors and high metabolic clearance [24,25]. These experiments show that the increase of hypothalamic NA content during the critical period of SBD induced by estrogens inhibiting CA *O*-methylation, is not enough for the disturbance of neuroendocrine control of ovulation without sufficient accompanying estrogenic influence. Taken together with our previous observations on similar effects of tropolone, a catechol-*O*-methyltransferase inhibitor [26], these data indicate a co-operative action of CA and brain-originated estrogenic metabolites of testosterone in androgen-dependent SBD.

Regarding TP-induced anovulation, data on the absence of the protective action of 6-OHDA, a neurotoxin for brain catecholaminergic receptors, are in agreement with evidence from other authors [27, 28]. However, they do not contradict the role of catecholamines in androgen-dependent SBD because, as our experiments have shown, 6-OHDA does not cause hypothalamic CA depletion in neonatally androgenized female rats at the end of the critical period of SBD.

We have found that maternal stress, similar to neonatal androgenization, eliminates sexual differences in CA content in the developing brain, however, these effects and those induced by neonatal androgenization are not alike. Alterations in CA content in discrete brain areas, induced by early influences, are not identical, and neither are their long-term consequences.

One of the major long-term manifestations of maternal stress is known to be demasculinization and feminization of sexual behaviour in male offspring [17], resulting from early alteration of the noradrenergic system in the POA, the regulatory center for male sexual behaviour, but not in the MBH, as we have shown. Obviously, anovulation and defeminization of sexual behaviour in neonatally androgenized rats is a result of the changes in NA content in the pup's MBH where functions of neural control of ovulation and female sexual behaviour in adulthood are located. Brain DA might be involved in the long-term effects of neonatal steroids and maternal stress.

In the perinatal period glucocorticoids determine the activity of tyrosine hydroxylase, a key enzyme of catecholamine synthesis, and attenuate the stress-reactivity of the pituitary–adrenal system in adulthood [29]. Hence the elevation of plasma corticosteroid levels in fetuses of stressed pregnant rats may be one of the possible mechanisms of modification of the noradrenergic system of the neuroendocrine complex in male pups and diminution of the stress-reactivity of the hypothalamic–pituitary–adrenal axis in adult offspring [30]. Another possible mechanism is a disorder of brain CA metabolism due to the slowing down of testosterone aromatization in male fetuses [31] and neonates [32] caused by prenatal stress. This hypothesis is supported by the data on the decrease of plasma testosterone levels in stressed fetuses and newborn male rats [33] as well as observations on the correlation between androgen aromatization in the hypothalamus and hypothalamic CA content at an early postnatal age [12].

Thus, it is concluded that early modification of brain CA by maternal stress and neonatal steroids are of considerable importance for the development of long-term neuroendocrine effects.

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