

**INTERACTION OF NEUROTRANSMITTERS:
GABAERGIC AND CHOLINERGIC PROCESSES**

The participation of monoaminergic central mechanisms on adenohypophyseal secretion

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In the hypothalamic area controlling the secretion of hypophyseal hormones [1-4] an high content of neurotransmitters [5-7] is present. However, their comparative significance in regulation of hypophysis-adrenal and hypophysis-gonadal systems is still not clearly established by the data in the literature, which report discrepant results on the activating or inhibitory influence of the various neurotransmitters [8-12]. At the same time it was shown that also the limbic structures (amygdala, septum, hippocampus) participate in regulation of secretion both of follicle-stimulating (FSH) and of luteinizing hormones (LH) [13, 14].

We report here on experiments carried out in rats, dealing with the action of drugs influencing the neurotransmitters on the functional activity of hypophysis-adrenal and of hypophysis-gonadal systems.

MATERIALS AND METHODS

The experiments were carried out in male rats. The full deafferentation of hypothalamus was realized by the method of Halasz and Pupp [15]. The activity of hypophysis-adrenal system was estimated by 11-oxy corticosteroids (11-OHCS) content in blood plasma determined fluorimetrically [16]. The level of catecholamines [17] and serotonin [7] in hypothalamus was also determined fluorimetrically. The microinjections of drugs in ventricles and structures of the brain were performed through steel cannulae chronically implanted according to the coordinates described by Szentagotai and Coll. [3]. Plasma LH content in rat was determined by the method of Parlow [18]. Electrolytic lesions of the brain limbic structures were made using a stereotaxic apparatus. The results were submitted to statistical analysis [19].

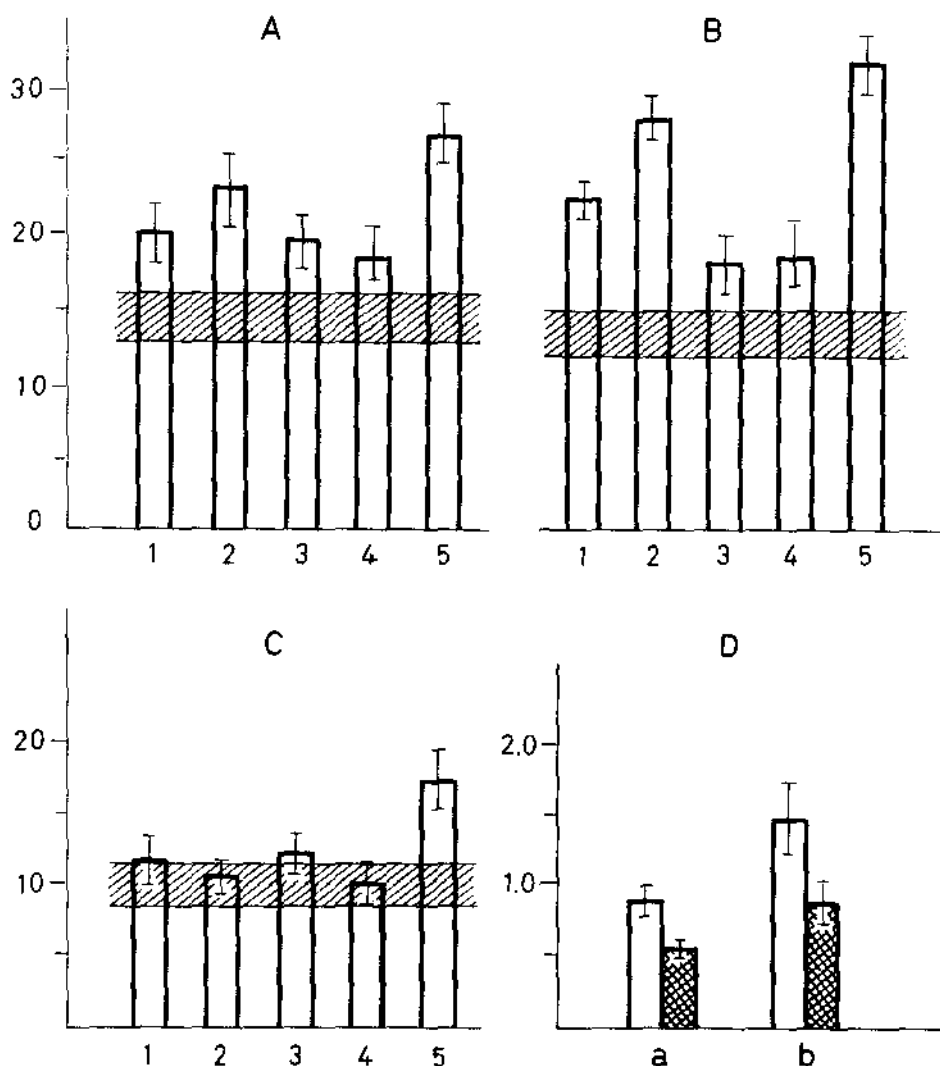


Fig. 1. — Influence of hypothalamic deafferentation on the plasma corticosteroids level ($\mu\text{g/g}$) and on the content of noradrenaline and serotonin in the brain ($\mu\text{g/g}$). *A* — intact animals; *B* — animals two weeks after full deafferentation of hypothalamus; *C* — rats treated by dexamethasone (1 mg/kg). Horizontal shaded line — 11-OHCS concentration in blood of control animals; columns — 11-OHCS concentration 30 min after injection in third ventricle of the brain of: 1 — noradrenaline (1 μg), 2 — serotonin (20 μg), 3 — arecoline (20 μg), 4 — histamine (20 μg), 5 — immobilization (15 min); *D* — content of noradrenaline (a) and serotonin (b) in the brain: light columns — control, dark columns — after hypothalamic deafferentation.

RESULTS AND DISCUSSION

In Fig. 1 are given the results of experiments in which the hypothalamus was isolated from afferent influences (hypothalamus deafferentation). The level of plasma corticosteroids in rats with isolated hypothalamus does not differ significantly from that of sham-operated animals.

Pretreatment with dexamethasone considerably decreases the stimulation of the hypophysis-adrenal system exerted by noradrenaline, serotonin, arecoline, histamine, injected into the ventricles (Fig. 1 C). The stimulating influence of these mediators is also observed in animals with hypothalamic deafferentation (Fig. 1 B). In these animals the reaction of hypophysis-adrenal system to the intraventricle microinjections of noradrenaline or serotonin and also to immobilization is higher in comparison with the sham-operated animals. This can be interpreted as an increased sensitivity of the deafferentated hypothalamus to these effects [27]. It should be noted in this regard that the content of noradrenaline and serotonin in hypothalamus is decreased after deafferentation (Fig. 1 D).

In further experiments, noradrenaline and serotonin content in the brain was correlated with plasma 11-OHCS concentration in different conditions (Fig. 2). Administration of α -methyl-*p*-tyrosine, microinjections in the lateral brain ventricles of 6-hydroxydopamine (6-OHDA), or coagulation of *locus ceruleus* are followed by a decrease of noradrenaline levels in hypothalamus. A decrease of serotonin content in the brain is observed after treatment with *p*-chlorophenylalanine, intraventricular administration of 5,6-dihydroxytryptamine, or electrocoagulation of the raphe nuclei. Lowering of noradrenaline and serotonin content in the brain is noted also after reserpine injection. However, all these changes do not influence significantly the corticosteroid concentration in blood, or the reaction of the hypophysis-adrenal system to the immobilization or to formaldehyde injection.

Thus, the excitation of the various neurohumoral brain systems leads to the activation of hypothalamus-hypophysis-adrenal system. This indicates a participation of these mediators in central regulation of corticotropic hypophysis hormones. However, the exhaustion of supply or blockade of synthesis of these mediators do not interfere with the rise of plasma corticosteroid levels in response to appropriate stimulations. It can be supposed that in these conditions the reaction of hypophysis-adrenal system is realized through the activation of subsidiary brain mechanisms.

In previous experiments [13] it was shown that electrolytic destruction of amygdala and of the dorsal part of hippocampus leads to inhibition of FSH and LH secretion, increase of sensitivity to the inhibitory estrogen

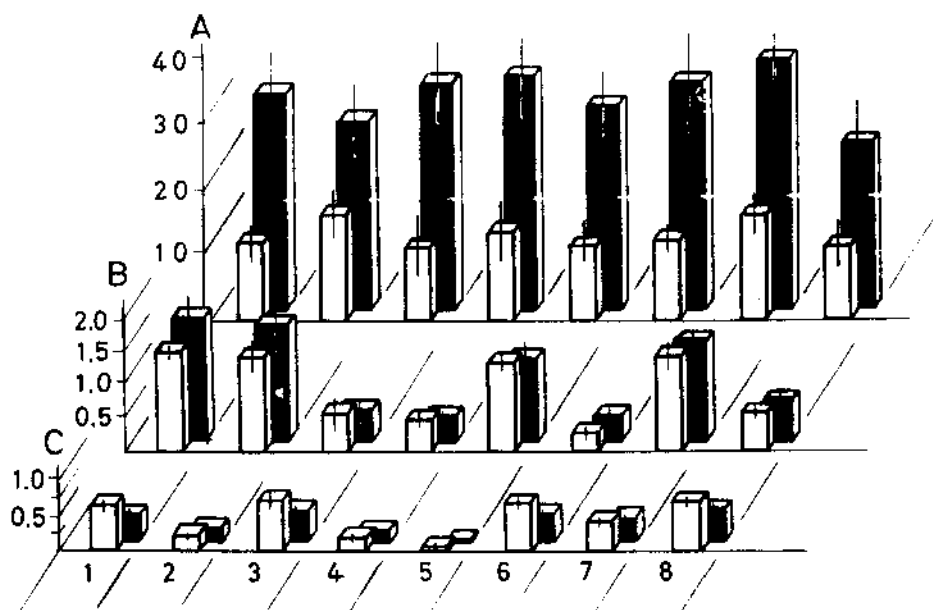


Fig. 2. — Content of plasma corticosteroids and level of biogenic amines in hypothalamus in different conditions. The concentration of 11-OHCS in $\mu\text{g}\%$ (A), content of serotonin (B) and noradrenaline (C) in the brain tissue in $\mu\text{g/g}$ are shown by columns: white columns — control, dark columns — after immobilization or after formaldehyde injection under aponeurosis (0.1 ml). 1 — isotonic solution (0.4 ml, intraperitoneally), 2 — α -methyl-*p*-tyrosine (150 mg/kg, intramuscularly, $\times 2$ days, 3 — *p*-chlorophenylalanine (400 mg/kg 35–40 hours before experiment, intramuscularly), 4 — reserpine (1 mg/kg 18 hours before experiment, intramuscularly), 5 — 6-OHDA (two injections in lateral ventricle of the brain at 24-hour intervals, 250 μg in 20 μl volume of 0.1% ascorbic acid solution, 10 days before experiment), 6 — 5,6-DHT (200 μg in lateral ventricle of the brain in 20 μl volume of 0.1% ascorbic acid solution, 10 days before experiment), 7 — 8 days after electrocoagulation of locus ceruleus bilaterally (2 mA, 15 sec), 8 — 8 days after electrocoagulation of raphe nuclei (2mA, 15 sec.).

action and blockade of spontaneous or hormone-induced ovulation. These changes are accompanied by a fall of noradrenaline content in hypothalamus (Table 1).

This is probably caused by destruction of the adrenergic pathways connecting limbic brain to the hypothalamus. Injection of estrogens induces a rise in noradrenaline hypothalamic levels of control animals, but does not affect noradrenaline level in animals with lesioned dorsal hippocampus. At the same time, plasma LH levels were increased in controls, while were decreased in animals with lesioned hippocampus.

TABLE 1

**Catecholamine content in hypothalamus
and plasma LH level after coagulation of some limbic brain structures**

GROUP	Adrenaline $\mu\text{g/g}$	Noradrenaline $\mu\text{g/g}$	Plasma LH $\mu\text{g/ml}$
Control	0.106 ± 0.01	0.83 ± 0.03	—
Destruction of hippocampus	0.107 ± 0.01	(a) 0.6 ± 0.02	—
Destruction of amygdala .	0.110 ± 0.02	(b) 0.42 ± 0.01	—
Control	0.07 ± 0.01	0.6 ± 0.02	0.4 ± 0.04
Estradiol monobenzoate 10 $\mu\text{g} \times 7$ d.	0.06 ± 0.01	(a) 0.8 ± 0.02	0.5 ± 0.05
Destruction of hippocampus	0.07 ± 0.02	(b) 0.46 ± 0.01	0.35 ± 0.03
Destruction of hippocampus + estradiol monobenzoate 10 $\mu\text{g} \times 7$ d. .	0.07 ± 0.01	0.44 ± 0.01	(b) 0.23 ± 0.02

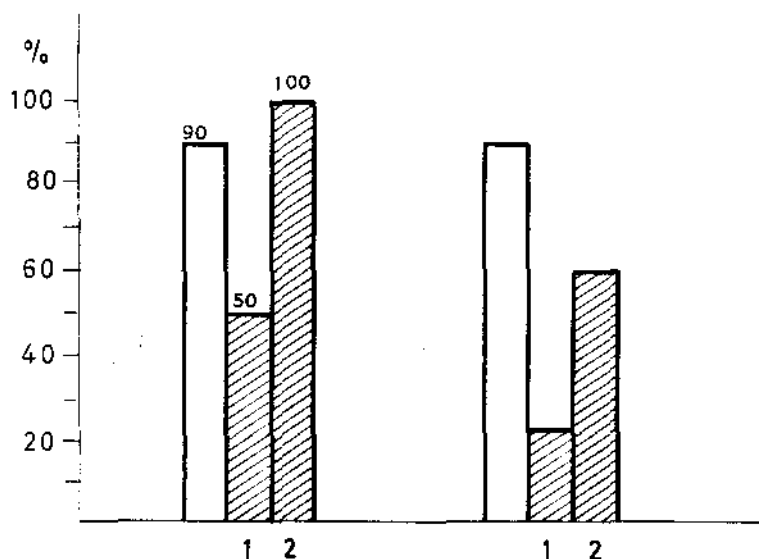
(a) $P < 0.02$.(b) $P < 0.001$.

Fig. 3. — Influence of 1-DOPA on rat ovulation. Percent of ovulated rats is shown by columns. Left columns: experiments with dorsal hippocampus destruction; right columns: experiments with the brain septum destruction. White columns: control; shaded columns: 1 — structure coagulation, 2 — structure coagulation and 1-DOPA (20 mg/kg for 7 days).

The connection between brain catecholamines content and level of gonadotropin secretion is confirmed by experiments with 1-dioxyphenylalanine (1-DOPA), α -adrenoblockers and β -adrenoblockers. 1-DOPA normalizes ovarian cycle in animals with disturbed ovulation after hippocampal destruction. Similar results following 1-DOPA are obtained after destruction of the brain septum (Fig. 3). The α - and β -adrenoblockers phentolamine hydrochloride and propranolol (10 μ g in 0.2 μ l volume for 7 days) were injected into the dorsal hippocampus. Phentolamine inhibited spontaneous ovulation, and hence, LH secretion, but had only a little influence on the ovulation induced by estradiol and by progesterone. Propranolol inhibited gonadotropic hypophysis function (showed by compensatory ovary hypertrophy test) and also decreased FSH content in the hypophysis.

The present results bring evidence that adrenergic mediation play a significant role in the mechanism of limbic influences on hypothalamic regulation of gonadotropin secretion.

Summary. — In acute and chronic experiments in rats has been studied the action of drugs influencing central neuro-transmitter on the functional activity of hypophysis-adrenal and hypophysis-gonadal system. Evidence has been obtained that central neurotransmitters participate in the control of ACTH-glucocorticoids secretion. However, there is no clear correlation between noradrenaline and serotonin level in the brain and the degree of reaction of pituitary-adrenal complex to stimuli.

Pharmacological analysis of nervous regulation of gonadotropic hypophysis function has revealed the significance of adrenergic mediation in the mechanism of feedback action of sex hormones and in the mechanism of limbic influences on hypothalamic regulation of gonadotropin secretion.

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