Effect of Serotonin on Gonadal Function

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The role of endogenous serotonin in the regulation of spermato- and oogenesis was studied in experiments on rabbits. The involvement of the peripheral serotoninergic structures in the studied processes is demonstrated.

Key Words: *spermatogenesis; oogenesis; serotonin*

Serotonin was detected in the vas deferens in rats. Its content increased with increasing the content of L-tryptophane (serotonin precursor). Serotonin increases contractile activity of smooth muscle strips from vas deferens [12], which attests to its serotoninergic innervation. Serotonin induced dose-dependent contraction of the vas deferens [4,5].

The motility of the vas deferens can be regulated via a purinergic mechanism. The presence of purinergic innervation of the reproductive system in male rats was demonstrated by immunohistochemical methods. P2×1 and P2×2 receptors were detected on smooth muscle cell membranes of the vas deferens, which suggested the involvement of these receptors in the vas deferens motility [10].

Serotonin caused effects similar to those of norepinephrine and dopamine, for example, contraction of isolated rat uterus and vas deferens [13,14].

Serotonin, dopamine, and norepinephrine play the leading role in the regulation of hypothalamic neurons secreting luteinizing hormone releasing factor. In addition, serotonin and NO stimulate gonadotropin and prolactin secretion interacting with 5-HT_1 and 5-HT_2 receptors located in the medial preoptic region. Serotonin regulates the levels of luteinizing (LH) and follicle-stimulating hormones (FSH). The hypothalamic nuclear serotonin mediates the regulation of ovarian hormonal activity and ovulation [1]. This was confirmed by the results of injections of 5-HT_1 receptor antagonist (methiotepine) and 5-HT_2 receptor antagonist (ketanserin) to ovariectomized rats injected with estrogen in combination with progesterone [8]. Estrogen stimulates, while ovariectomy reduces the expression of 5-HT_{1A} receptors by cells in the hypothalamic ventromedial nucleus [7].

The serotoninergic system and neuroactive steroids are in reciprocal relationships. On the other hand, during gestation the stimulatory activity of serotoninergic neurons increases in parallel with the increase in progesterone content [11].

Sex steroids and gonadotropins are essential for normal stermatogenesis. Testosterone triggers this process by modulating the spermatogonia and stimulating meiotic division of primary spermatocytes, this resulting in the formation of secondary spermatocytes and young spermatids. Maturation of spermatids to spermatozoa is realized under FSH control. Injection of serotonin reuptake inhibitor venlafaxine increases the content of FSH [9]. However, the role of neurotransmitter serotonin in the regulation of female and male gonadal functions remains not quite clear.

We studied the role of neurotransmitter serotonin in spermato- and oogenesis.

MATERIALS AND METHODS

Experiments were carried out on 16 Chinchilla rabbits (3-4 kg) under Nembutal narcosis (40 mg/kg). The peripheral fragment of the right vagus nerve was stimulated with rectangular current pulses of 1-10 V amplitude, 5-10 Hz frequency, 2 msec du-

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ration; peripheral fragment of the left sympathetic trunk was stimulated with rectangular pulses of 2.5-12.5 V amplitude, 5-10 Hz frequency, and 2 msec duration. Intact rabbits served as controls. The electromotor activity (EMA) of the vas deferens was recorded using bipolar silver electrodes with contact surface area of 1.5-2.0 mm^2 and 1.5 mm distance between the poles. Analysis of EMA included measurement of the amplitude and frequency of EMA slow waves and spike activity (expressed by the number of spikes/100 EMA slow waves). In control series the vagus nerve and sympathetic trunk were stimulated separately or the vagus stimulation was supplemented by stimulation of the sympathetic trunk. Pharmacological analysis of the detected phenomenon was carried out in the next 4 series of experiments with α- and β-adrenoblockers (phentolamine and propranolol), purinergic receptor blocker (theophylline) in a dose of 20-80 mg/kg, 5-$HT_{3,4}$-receptor blocker (droperidol; 0.5-1.0 mg/kg), and 5-$HT_{1,2}$-receptor blocker (sumatryptane; 0.5-1.0 mg/kg).

Male and female gonadal functions were evaluated by morphological criteria before and after nerve stimulation. Ovarian and testicular biopsy specimens were fixed in 100% formalin, dehydrated in ascending alcohols, and embedded in paraffin. Histological sections were stained with hematoxylin and eosin.

RESULTS

Control series of experiments showed that stimulation of the peripheral fragment of the right vagus nerve led to activation of the vas deferens EMA slow waves (Fig. 1, *a*). Summary results of experiments showed that basal frequency of EMA slow waves was 9.3±1.8/min, the amplitude 0.20±0.02 mV; stimulation of vagus nerve led to an increase in EMA slow wave frequency to 13.2±1.9/min (42%, $p<0.05$) and of the amplitude to 0.23±0.05 mV (15%).

Stimulation of the sympathetic trunk after stimulation of the vagus nerve potentiates the vagal stimulatory effect on the motility of the vas deferens: slow wave frequency reached 18.4± 2.3/min (40%, $p<0.05$), amplitude to 0.27±0.05 mV (17%).

Hence, increase of the vagus stimulation of the vas deferens EMA by the sympathetic nerve stimulation is observed when the right vagus and left sympathetic nerves on the animal neck are stimulated. The studied phenomenon was detected against the background of intact β- and α-adrenoceptors in rabbits weighing 3-4 kg and medium-intense stimulation of the vagus nerve ("vagus optimum").

The study of the possible involvement of α- and β-adrenoceptors in the mechanism of sympathetic amplification of vagus stimulation of the vas deferens motility in the next experimental series showed that the basal frequency of EMA slow waves before nerve stimulation was 7.6±1.2/min and amplitude 0.21±0.03 mV. Stimulation of vagus nerve increased EMA slow wave frequency to 1.8±1.3/min (55.2%, $p<0.05$) and amplitude to 0.22±0.02 mV (5%).

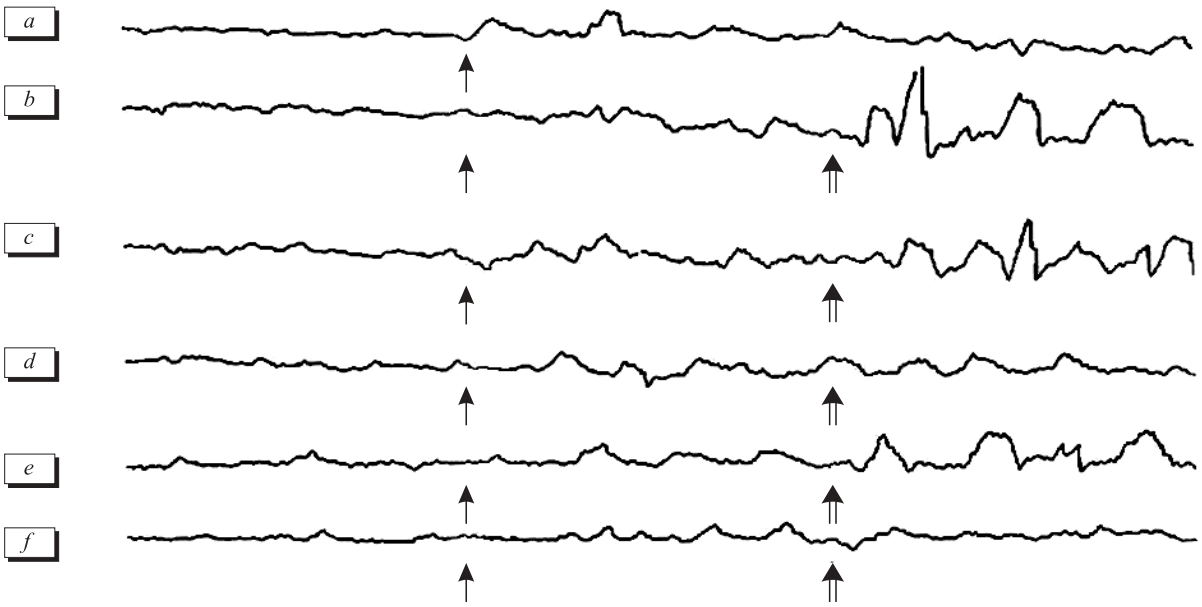

Fig. 1. Vas deferens EMA after stimulation of the vagus nerve (*a*), co-stimulation of the sympathetic trunk and vagus nerve before (*b*) and after theophylline injection (*c*), after droperidol (*d*), before (*e*) and after sumatriptan injection (*f*). Arrow shows stimulation of vagus nerve, double arrow shows co-stimulation of sympathetic trunk and vagus nerve. Vertical scale: 0.2 mV; horizontal scale: 5 sec.

Stimulation of the sympathetic trunk after vagus stimulation (Table 1) further increased the EMA slow wave frequency to 14.9±1.3/min (22%, $p<0.05$) and the amplitude to 0.23±0.03 mV (4%). Basal frequency of EMA slow waves after propranolol and phentolamine blockade of β- and α-adrenoceptors was 7.30±0.03/min, at 0.23±0.02 mV amplitude. After vagus stimulation EMA slow wave frequency increased to 13.8±1.2/min (75%, $p<0.05$) in the presence of stable amplitude. Further stimulation of the sympathetic trunk against the background of vagus stimulation additionally increased the EMA slow wave frequency to 16.8±1.4/min (21%, $p<0.05$) and the amplitude to 0.25±0.03 mV (8%).

Hence, injection of α- and β-adrenoblockers did not cancel the sympathetic potentiation of vagus stimulation of the vas deferens EMA. Hence, the adrenergic structures are not the key ones in the realization of the studied phenomenon.

Possible involvement of purinergic structures in the mechanism of the studied effect realization was studied in series III of experiments. Before theophylline injection, the stimulation of the sympathetic trunk in parallel with vagus nerve stimulation led to a 40% increase in the vagus stimulatory effect of the vas deferens EMA (from 13.2±1.9 to 18.5±2.3/min). After theophylline injection, the basal frequency of EMA slow waves was 6.3±0.3/min, amplitude 0.20±0.02 mV. After vagus nerve stimulation the slow wave frequency increased to 11.0±1.3/min (77%, $p<0.05$) and the amplitude to 0.23±0.02 mV (15%). Sympathetic trunk stimulation against the background of vagus nerve stimulation amplified the vagus stimulatory effect to 17.6±3.8/min (60%, $p<0.05$) in the presence of stable amplitude (Fig. 1, *b*, *c*).

Our results indicate that blockade of purinergic systems not only failed to remove the studied effect, but even amplified it.

Possible involvement of serotoninergic structures during synergic effects of the vagus and sympathetic nerve on the vas deferens EMA was studied in series IV of experiments. Possible involvement of the ganglion 5-$HT_{3,4}$-serotonin receptors in the mechanisms of the studied effect realization was studied using 5-$HT_{3,4}$-blocker (droperidol) (Fig. 1, *d*).

Before droperidol injection, the basal frequency of EMA slow waves was 6.2±0.3/min, amplitude 0.20±0.02 mV. Stimulation of vagus nerve increased slow wave frequency to 11.0±1.3/min (93%, $p<0.05$), amplitude reached 0.23±0.04 mV (15%). Further stimulation of the vagus nerve increased slow wave frequency to 17.6±3.8/min (60%, $p<0.05$) in the presence of stable amplitude. After injection of droperidol, stimulation of the vagus nerve resulted in an increase of the basal frequency of the vas deferens EMA slow waves from 9.0±0.8 to 12.0±1.2/min (30%, $p<0.05$). Subsequent stimulation of the sympathetic trunk after vagus nerve stimulation did not amplify the vagus stimulatory effect: EMA slow wave frequency was 12.0±1.5/min.

Hence, the results indicate that intramural ganglia with 5-$HT_{3,4}$-receptors on their surface are involved in the realization of the studied effect.

The final series of experiments investigated the possibility of 5-$HT_{1,2}$-serotonin receptors involvement in the realization of the studied effect. Before injection of sumatriptan (5-$HT_{1,2}$-receptor blocker), the vas deferens EMA slow wave frequency was 11.0±2.5/min, amplitude 0.20±0.01 mV. Stimulation of the vagus nerve increased EMA slow wave frequency to 16.0±2.0/min (45%, $p<0.05$). Stimulation of the sympathetic trunk in parallel with stimulation of the vagus nerve promoted an increase in the EMA slow wave frequency to 23.5± 3.5 min (47%, $p<0.05$). After injection of sumatriptanthe, basal frequency of the vas deferens EMA slow waves was 9.0±1.5/min, amplitude 0.18±0.03 mV. Stimulation of the vagus nerve led to increase in basal frequency of slow waves to 13.5±1.5/min, (50%, $p<0.05$); subsequent stimulation of the sympathetic trunk in parallel with vagus stimulation not only blocked the effect, but even decreased EMA slow wave frequency to 11.0±1.0 (18%), with the EMA

TABLE 1. Drug Effects on Sympathetic Amplification of Vagus Nerve Stimulation of the Vas Deferens EMA (*M*±*m*)

Drug	Before injection				After injection			
	basal	vagus stimulation	vagus+ sympathetic stimulation	%	basal	vagus stimulation	vagus+ sympathetic stimulation	%
Phentolamine+propranolol	7.6±1.2	11.8±1.3	14.9±1.3	22	7.3±0.3	13.8±1.2	16.8±1.4	21
Theophylline	7.8±1.1	13.2±1.9	18.5±2.3	40	6.3±0.3	11.0±1.3	17.6±3.8	60
Droperidol	6.9±0.3	11.0±1.3	17.6±3.8	60	9.0±0.8	12.0±1.2	12.0±1.5	0
Sumatryptane	11.0±2.5	16.0±2.0	23.5±3.5	47	9.0±1.5	13.5±1.5	11.0±1.0	-18

slow wave amplitude gradually decreasing from 0.18±0.03 mV at the beginning of nerve stimulation to 0.15±0.05 mV during co-stimulation of the sympathetic trunk and vagus nerve (Fig. 1, *e*, *f*). Hence, injection of 5-$HT_{1,2}$-serotonin blocker (sumatryptane) completely blocked the studied effect (Fig. 1, *f*).

Photooptic study of ovarian structure in control rabbits showed common morphology of the ovarian cortex and medulla, corresponding to their histoarchitecture (Fig. 2, *a*-*d*).

Various stages of follicle maturation are presented: growing subcapsular follicle and its rupture (Fig. 2, *e*). The follicle was ruptured under the effect of LH, because of activation of its production by the pituitary. Our previous studies [2] showed that stimulation of the sympathetic trunk in parallel with stimulation of the vagus nerve led to activation of preganglionic serotoninergic fibers, through

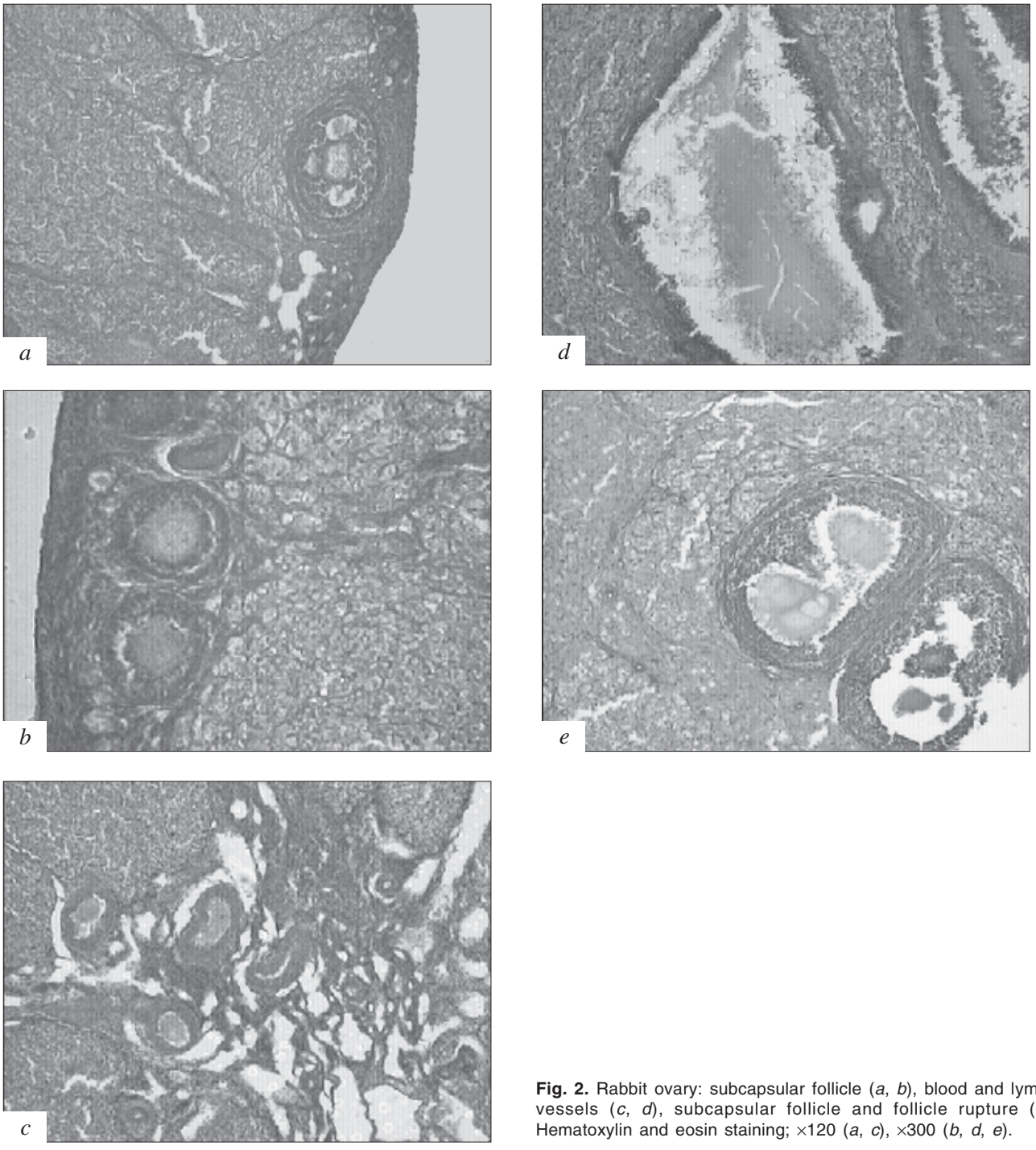

Fig. 2. Rabbit ovary: subcapsular follicle (*a*, *b*), blood and lymph vessels (*c*, *d*), subcapsular follicle and follicle rupture (*e*). Hematoxylin and eosin staining; ×120 (*a*, *c*), ×300 (*b*, *d*, *e*).

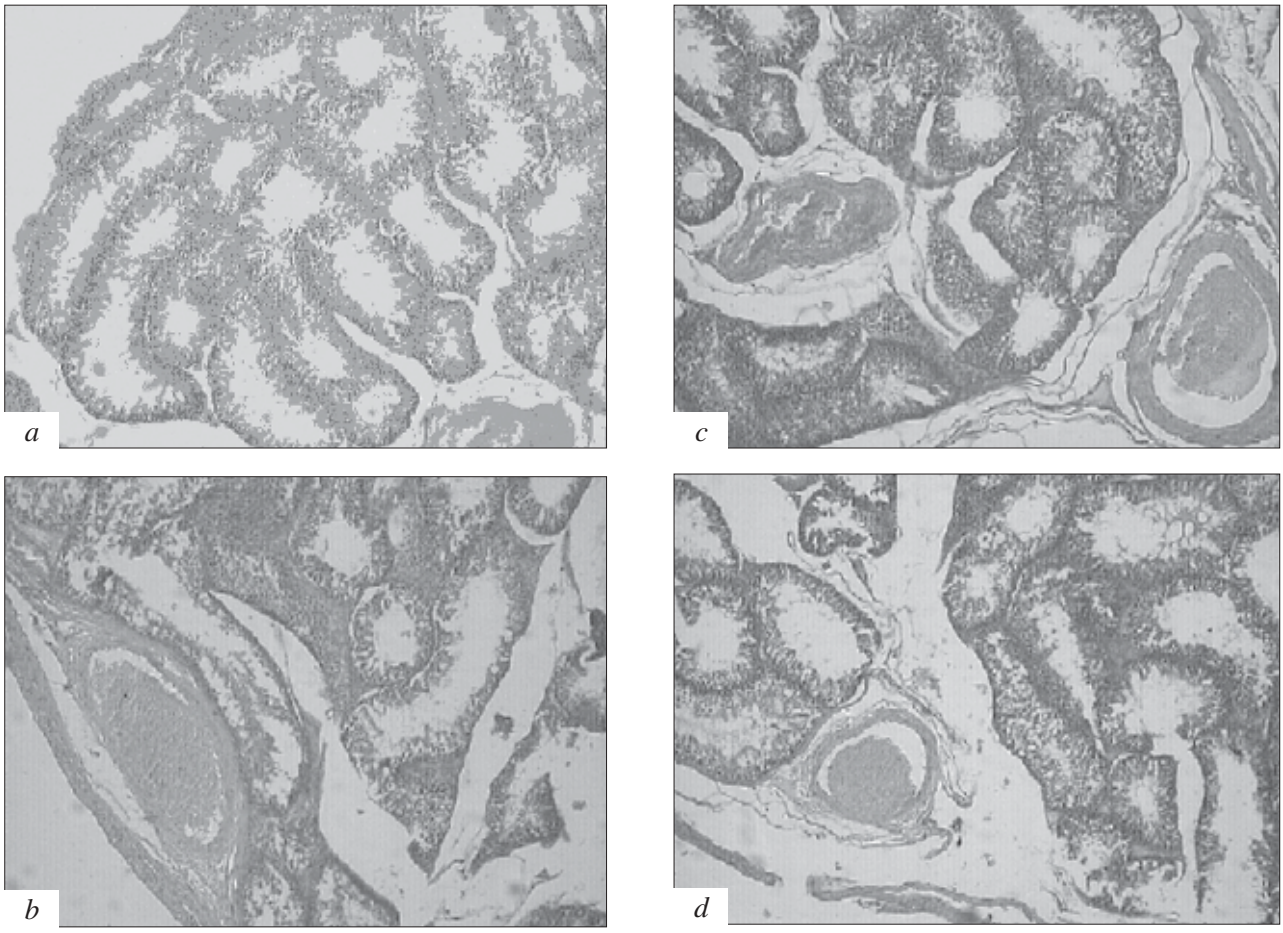

Fig. 3. Rabbit vas deferens (*a*, *b*), testes and vessels (*c*, *d*) in health (*a*, *c*) and hyperserotoninergic syndrome (*b*, *d*). ×120 (*a*, *b*, *d*); ×250 (*c*).

which stimulation was transferred to the ganglionic serotoninergic neurons, activating serotonin receptors of effector tissues (heart, stomach, duodenum, gallbladder and Oddi's sphincter, large intestine, urinary bladder and ureters, uterus and uterine tubes). The serotoninergic system is activated after single simultaneous stimulation of the sympathetic and parasympathetic nerves and persists during periodically repeated (for 15-17 h) parallel stimulation of the nerves, which leads to the development of the hyperserotoninergic syndrome.

Serotonin stimulates the formation of LH, which activates oogenesis (Fig. 2, *e*) and spermatogenesis. The presence of ruptured folliculi is an evidence of oogenesis activation, because normally they are not seen in female rabbits during the estrus phase I (the phase of females under conditions of isolation). The development of hyperserotoninergic syndrome is associated with ovulation and activation of the uterine tubes motility (endogenous serotonin accelerates ovulation and stimulates the motor activity of the uterine tubes, which promotes migration of a mature oocyte into the uterine cavity).

Photooptic examination of the vas deferens transverse section showed no pathological changes. The vas deferens was lined with two-row epithelium, whose cells exhibited signs of secretory activity. The vas deferens mucosal epithelium lay on the basal membrane separating it from the lamina propria, consisting of connective tissue abundant in elastic fibers. The muscular membrane was formed from circular bundles of smooth muscle cells, with a nerve plexus containing ganglionic cells in the thickness of these bundles. The vas deferens was coated with a connective tissue adventitial membrane (Fig. 3, *a*).

The structure of the rabbit testis, according to photooptic findings, corresponded to the normal: fine connective tissue septae, originating from the external tunica albuginea, divided the parenchyma of the testis into lobules, each containing up to two twisted tubules, in which spermatogenesis took place. The spermatic tubule cavity was lined with a layer of supporting cells, with several rows of spermatogenic epithelium (located on the basal membrane) among them. Bundles of collagen fibers were located circularly and longitudinally, with elastic fi-

bers between them; supporting cells of twisted seminal tubules in adult animals were connected by their processes (Fig. 3, *c*).

The hyperserotoninergic syndrome was associated with a lesser height of the two-row epithelium of the vas deferens (Fig. 3, *b*), presumably because of excessive secretory activity. It was detached from the basal membrane at some sites. The veins were dilated. Hypersecretion of endogenous serotonin leads to activation of spermatogenesis, which was seen from the initial signs of spermatogenic epithelium exhaustion (Fig. 3, *d*).

Ruptured follicles were detected in rabbits with ovarian hyperserotoninergic syndrome; these ruptures could result from the stimulatory effect of serotonin on LH synthesis and secretion.

Electrophysiological studies showed that potentiation of vagus stimulation of the vas deferens EMA by the sympathetic nerve was realized through activation of preganglionic serotoninergic fibers, transmitting stimulation to the ganglionic neuron 5-$HT_{3,4}$-serotonin receptors, activating the effector cell 5-$HT_{1,2}$-serotonin receptors.

Comparative morphological study showed intensification of secretory activity of the vas deferens epithelial cells in hyperserotoninergic syndrome and thinning of the spermatogenic epithelium in the testes. Dilated veins were detected in all studied tissues of animals with the hyperserotoninergic syndrome; it is a characteristic morphological sign of serotonin effect on the smooth muscles of effector tissues.

The results of electrophysiological and morphological studies suggest that the hyperserotoninergic status is associated with intensification of oo- and spermatogenesis and the motility of the vas deferens and uterine tubes, resulting in more rapid fertilization of the oocyte.

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