MORPHOLOGICAL AND FUNCTIONAL EFFECTS OF PYRACRYL ON FEMALE REPRODUCTIVE ORGANS IN RATS

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UDC 615.23.03:616.24-003.662].015.4:612.62].076.9

Key Words: pyracryl; ovaries; uterus; tissue basophils.

The search for therapeutic preparations with effective antisilicotic properties is an urgent task in biology and medicine. Piracetam (2-pyridylethylmethacrylate-phosphate) under experimental conditions is known to possess such an action [1]. However, its clinical use for the treatment of silicosis and other types of pneumoconiosis, accompanied by the development of fibrosis of the lung, is possible only if side effects are absent. The effect of pyracryl on organs of the female reproductive system has virtually not been studied from this standpoint. As we know, besides the pituitary gonadotrophic hormones, an important role in the regulation of their functional activity is played by biogenic amines and other vasoactive hormones of the tissue basophils. Histamine and serotonin, secreted by them, regulate the blood flow and vascular permeability and allow gonadotrophins, water, and salts [2, 7, 8] and also, possibly, xenobiotics, including therapeutic preparations, and chemicals which have entered the internal medium of the body, into the tissues. Some of them may have a trigger mechanism on the tissue basophils, causing them to secrete biologically active substances. We also know that an excess of serotonin in the tissues of the reproductive organs gives a gonadotrophic effect [4]. This suggests that this effect may be the result both of the direct action of the chemical substance on structural elements of the ovaries and uterus and of a direct effect of biologically active substances and hormones secreted by the tissue basophils. A third pathogenetic mechanism likewise cannot be ruled out: their combined action.

The aim of this investigation was to study the possible gonadotrophic effects of pyracryl and their connection with the functional activity of the tissue basophils of the female reproductive organs.

EXPERIMENTAL METHOD

Experiments were carried out on 63 female Wistar rats weighing 220-240 g. Pyracryl was injected intramuscularly into the experimental animals in therapeutic doses of 1 and 5 mg/kg daily for 1 month. The rats in the stage of estrus were killed by decapitation 1 month after the beginning of injection of the drug (subacute period) and 1 month after the end of its administration (recovery period). The state of ovarian and uterine function was assessed by the duration of the estrus cycle in each of its stages. Pieces of the organs for histological investigation were fixed in 10% neutral formalin solution, dehydrated in alcohols of increasing strength, and embedded in paraffin wax. Serial sections were stained with hematoxylin and eosin. In the ovaries the number of primary, secondary, tertiary, and atretic follicles and the number of corpora lutea were counted. A combined morphometric analysis of functional activity of the tissue basophil populations in the ovaries and uterus also was undertaken. They were demonstrated by staining sections with basic brown by the method in [5, 6]. The morphological and functional analysis of the tissue basophil population comprised determination of the following parameters: the composition of the cell profiles for different phases of the secretory cycle, determined as the number of secretory granules in the cell and the intensity of their staining; calculation indices of degranulation and saturation of the cells by secretory granules and counting the number of tissue basophils in 1 mm^2 area of section [3]. The quantitative parameters obtained were subjected to statistical analysis on a "Hewlett-Packard 9825A" personal computer.

Laboratory of Pathomorphology and Electron Microscopy Group, Research Institute of Work Hygiene and Occupational Diseases, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR M. R. Sapin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 5, pp. 508-510, May, 1990. Original article submitted April 30, 1989.

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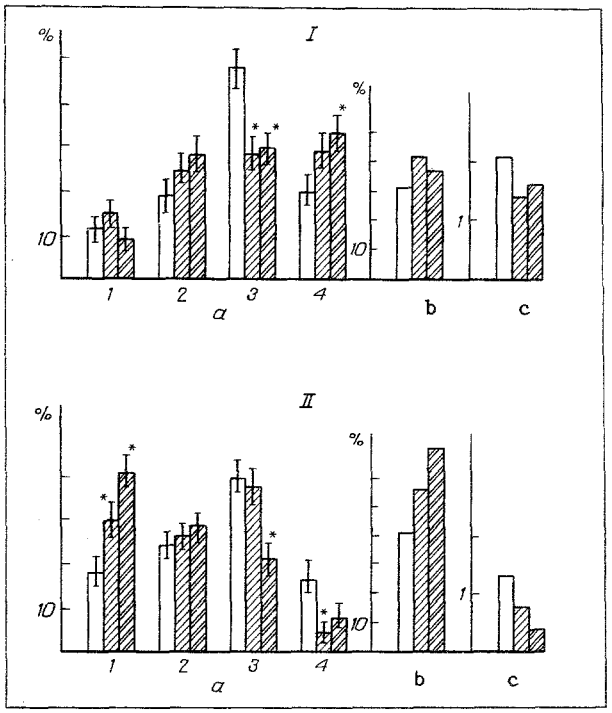

Fig. 1. Morphometric criteria of state tissue basophil population in rat ovary during treatment with pyracryl. Here and in Fig. 2: I) subacute period, II) recovery period; a) cytograms of tissue basophil population (in %): 1) very pale, 2) pale, 3) dark, 4) very dark tissue basophils; b) degranulation index of tissue basophils; c) saturation index of tissue basophils. Unshaded columns — control, single oblique shading 1 mg/kg, double oblique shading 5 mg/kg.

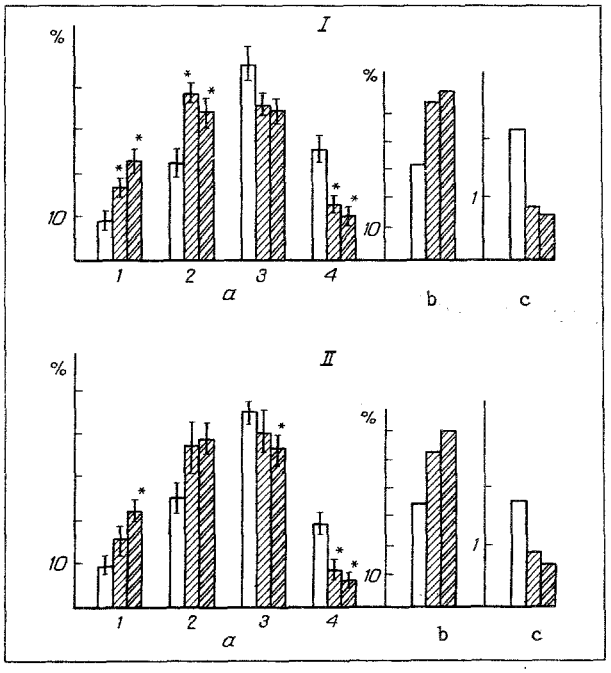

Fig. 2. Morphometric criteria of state of tissue basophil population of rat uterus under the influence of pyracryl. Legend as to Fig. 1. $*p < 0.05$.

TABLE 1. Number of Tissue Basophils per Unit Area in Ovary and Uterus of Albino Rats Receiving Pyracryl

Organ	Group	Period	
		substance	recovery
Ovary	Control	7,1±1,9	15,2±4,3
	1 mg/kg	20,7±5,6*	12,8±5,0
	5 mg/kg	17,7±4,2*	21,6±4,6
Uterus	Control	1, ±0,28	0,86±0,06
	1 mg/kg	2,4±0,36*	0,82±0,11
	5 mg/kg	2,2±0,40*	1,06±0,11

Legend. *$p < 0.05$.

EXPERIMENTAL RESULTS

Injection of pyracryl into rats in doses of 1 and 5 mg/kg daily for 1 month caused dilatation and congestion of the vessels of the microcirculatory bed, edema of the tissues, and lymphostasis in the ovaries and uterus. These changes were observed against the background of infiltration of the connective tissue of the organs by tissue basophils.

It will be clear from Table 1 that injection of pyracryl in both doses caused an increase in the number of tissue basophils per unit area by 2.5-3 times compared with the control group. The number of dark forms of tissue basophils in this case was reduced and the number of pale forms increased; the denaturation index was increased and the index of their saturation with secretory granules was reduced (Figs. 1 and 2). These parameters indicate considerable release of secretory product, containing histamine, serotonin, and other vasoactive hormones, by the tissue basophils. Morphometric analysis of the ovaries of the animals receiving pyracryl in a dose of 5 mg/kg revealed an increase in the number of atretic follicles (1589 ± 159 compared with 1010 ± 181 in the control, $p < 0.05$). In the animals of this group, an increase in the duration of the estrus cycle also was observed (4.5 ± 0.2 compared with 2.8 ± 0.2 days in the control, $p < 0.05$) on account of the estrus stage.

Changes of a rather different character were observed in animals receiving pyracryl in a dose of 1 mg/kg. An increase in the number of corpora lutea (15.2 ± 1.0 compared with 11.1 ± 1.0 in the control, $p < 0.05$) was found in the ovaries of the albino rats of this group, whereas their estrus cycle followed a more normal course.

In the recovery period many parameters of the morphological and functional state of the ovaries and uterus reached levels found in animals of the control group. Infiltration of the tissues of the ovaries and uterus by tissue basophils disappeared (Table 1). Meanwhile, pale degranulated forms still predominated among them (Figs. 1 and 2), evidence of increased secretion of biogenic amines and other biologically active substances by them.

The results thus show that the tissue basophils of the female reproductive organs are sensitive to pyracryl. Injection of this substance for 1 month in doses of 1 and 5 mg/kg led to infiltration by tissue basophils and their marked degranulation in the ovaries and uterus, with disturbance of the microcirculation of blood and lymph. This gonadotrophic effect is evidently the result of the combined action of pyracryl and of biologically active substances secreted by the tissue basophils. Nevertheless, a decisive role in the pathogenetic mechanisms of the gonadotrophic effect is played by the action of pyracryl. This conclusion is supported by the fact that injection of pyracryl in a dose of 1 and 5 mg/kg caused equal infiltration of the tissues of the ovaries and uterus by tissue basophils and their marked degranulation. However, the gonadotrophic effects were qualitatively different depending on the dose of the drug given. For instance, pyracryl in a dose of 1 mg/kg stimulates follicle formation, as shown by an increase in the number of corpora lutea in the ovaries. Conversely, injection of the drug in a dose of 5 mg/kg caused marked atresia of the growing follicles and disturbance of the course of the estrus cycle. These data are exceptionally important for the pharmacopeal assessment of pyracryl as a therapeutic preparation. Meanwhile, the results so far obtained show that the pathogenetic mechanisms of the gonadotrophic effects of chemical substances are complex, and their study requires analysis of the morphological and functional state of the tissue basophils of the female reproductive organs.

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ULTRASTRUCTURAL DETECTION OF CHOLESTEROL IN THE LIVER OF ALCOHOLIC RATS

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UDC 616.89-008.441.13-092-07: 616.36-008,939.22-07

Key Words: hepatocyte ultrastructure; cholesterol; alcohol

Disturbance of lipid metabolism is one of the main features of liver disease. At the hepatocyte level this is manifested as accumulation of lipids and changes in the intracellular organelles [1, 7, 8]. Deposition of cholesterol in the liver is one form of disturbed lipid metabolism. There are few reports in the literature on this subject [2, 5, 6]. The aim of this investigation was to study the distribution of cholesterol in hepatocytes and to determine its effect on the state of the intracellular ultrastructures in the liver during alcohol poisoning.

EXPERIMENTAL METHOD

Cholesterol deposition was studied in 35 male albino rats weighing 180-220 g, receiving alcohol in a dose of 2 g/kg body weight by the intragasric route. The liver was studied electron-microscopically 1, 7, 14, 30, and 45 days after the beginning of alcohol administration. The control group consisted of 15 intact animals. For a general electron-microscopic survey the liver tissue was treated by the usual method. Electron-cytochemical demonstration of free cholesterol was undertaken by a reaction based on the formation of a cholesterol–digitonin complex [9, 10]. We developed a perfusion method of fixing the liver and incubating it in situ: under ether anesthesia laparotomy was performed and the liver perfused through the portal vein. To maintain the necessary pressure in the system, a perfusion apparatus [4] was used. The indicator of successful perfusion was a uniform pale yellow color of all the lobes of the liver. For prefixation, a 4% glutaraldehyde-formaldehyde fixing solution, made up in Hanks' buffer, pH 7.2, at 20°C was used for 5 min (70-150 ml of solution, or on average 5 ml/min/100 g body weight). Immediately after prefixation, the liver was perfused for 5 min with incubation medium (50-70 ml) containing pH 7.2, at 20°C (full details of the method of preparing the solutions are given by Loginov et al. [3]). After incubation the liver tissue, cut up into pieces measuring 1 mm^3, was transferred to a fresh portion of 0.2% digitonin for 4 h or allowed to stand overnight at 4°C. The specimens were then washed with 0.1 M cacodylate buffer (pH 7.2) and postfixed in 1% OsO_4 in cacodylate buffer for 2 h. The following schedule of dehydration and embedding in Araldite was used: 30-70° alcohol 5 min, 96° alcohol (three changes, 5 min each), saturation with a working mixture of M Araldite: N Araldite (1:1) with 2% catalyst for 2 h at room temperature. In the final stage the fragments were transferred into a fresh portion of the working mixture for 2 h at 37°C, after which they were embedded in Araldite and kept for 2 days at 60°C. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in the JEM-1200 EX electron microscope. The instrumental magnification was 5000-50,000.

Central Research Institute of Gastroenterology, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 5, pp. 510-512, May, 1990. Original article submitted April 8, 1989.

0007-4888/90/0005-0679$12.50