The diminished proliferation of granulosa cells in follicles at late preantral stages probably leads subsequently to a decrease of the mean number of granulosa cells in antral and preovulatory follicles and in a disruption of the physiology of the cell-cell relationships between granulosa and theca follicles. This manifests itself, in our opinion, in the cystoatresic transformation of a number of antral follicles observed in slides of ovaries of experimental animals against the background of a diminished population of "healthy" antral follicles (Table. 1).

Thus, hyperprolactinemia induced experimentally disrupts the physiological estrous cycle and causes anovulation in virtually 100% of cases.

The presumed onset of folliculogenesis "destruction" under hyperprolactinemic conditions occurs in the late preantral stages of development of the follicles, in whose membrana granulosa the mitotic activity of cells drops when the prolactin concentration is high. This may be due in some cases to a drop of the concentration of FSH (a natural mitogen for granulosa cells) as well as to possible prolactin inhibition of the aromatose system and, accordingly, of the intrafollicular synthesis of estradiol - another mitogenic factor for granulosa cells.

REFERENCES

1. G. F. Erickson, *Clin. Obstet. Gynecol.*, **21**, № 1, 90-120 (1978).
2. A. N. Hirshfield, *Biol. Reprod.*, **28**, № 2, 271-278 (1983).
3. A. N. Hirshfield, *Ibid.*, **31**, № 1, 52-58 (1984).
4. S. Lintern-Moor and A. V. Everitt, *Ibid.*, **19**, 188-191 (1978).
5. J. Wood, *Proc. Soc. Exp. Biol.*, **187**, № 4, 520 (1987).

The State of the Microcirculatory Bed, Microhemodynamics, and Oxygen Supply of the Liver under Conditions of Disrupted Parasympathetic Innervation

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Vagotomy is shown to result in disturbances of the microcirculation (a reduced rate of blood flow and distortion of its kinetics), the morphological basis of which consists of certain transformations of the microvascular network organization and ultrastructural changes in the cells lining the sinusoidal capillaries. The most pronounced disorders in microhemodynamics and blood supply of the liver are found 5-14 days after vagotomy.

Key Words: *liver; vagotomy; microcirculation; oxygen; hypoxia*

The mechanisms underlying the hypoxic state developing in organs of the digestive system in parasympathetic innervation disorders need to be specified. The data available are devoted, as a rule, to some particular aspect of the microcirculation (morphological, physiological, or biophysical [6,9, 11,14], and this hampers attempts to gain a comprehensive idea of all the regular transformations tacking place in the microhemodynamic system when the innervation is impaired and to judge the

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nature of the hypoxia occurring. The present investigation is a combined morphophysiological study of the microhemodynamics and oxygen supply of the liver and the structural foundations of these processes after resection of the vagus nerves. The following aims were established: 1) to study the structural organization of the liver microvascular bed (primarily its metabolic component) in various periods of the postvagotomic syndrome; 2) to examine the ultrastructure of the main components of the histohematic barrier; 3) to determine the basic kinetic parameters of the microcirculation; 4) to assess the state of the oxygen supply of the organ parenchyma; 5) to reveal possible correlations between the indexes studied.

MATERIALS AND METHODS

Experiments were carried out with 76 outbred male white rats with an initial weight of 180-210 g. Bilateral subdiaphragmatic vagotomy was performed in 42 animals under ether anesthesia, while the others (including 9 sham-operated rats) were the control. The local circulation rate (LCR) and oxygen tension (pO_2) in the liver parenchyma were measured with polarography (LCR according to H_2 clearance - 13, pO_2 - 2) in animals under urethane anesthesia (1.5 g/kg) in an acute experiment 16-18 h after the last feeding 5, 7, 14, 30, 60, and 90 days postoperation. Since the processes of tissue H_2 uptake and release are mainly governed by the local blood flow, we obtained the information on the circulatory kinetics via mathematical analysis of H_2 uptake-release curves resulting from LCR measurement. For this purpose the ratio of the amplitude A_i at time point t_i to the maximal amplitude A_{max} (every 0.25 min) was calculated manually both for the H_2 uptake phase (phase I) and for the release phase (phase II). Regression analysis was performed to reveal the relationship between A and t [1]. Since the nature of the dispersion diagrams in all experimental series pointed to a possible exponential dependence between A and t, the method of nonlinear regression was used for analysis of the initial data. The mathematical model in this case is specified by an exponential function with three parameters p_1, p_2, and p_3: $f_i(t_i,p_1,p_2,p_3)=p_1\times\exp(t/p_2)+p_3$. The model parameters were estimated by the method of iteration using the nonlinear regression program. Since parameters p_1 and p_3 are not essential for describing the kinetics of the processes studied but are of significance only for positional standardization of the corresponding curves on a plot, they do not figure among the results given. For morphometry (light microscopy) pieces from the left lobe of the liver were fixed in 10% buffered Formalin. The area of the sinusoidal capillaries was determined on paraffin sections stained with hematoxylin-eosin using an Avtandilov grid. For electron microscopic examination organ samples were fixed in 2.5% glutaraldehyde with subsequent postfixation in osmium tetroxide, dehydrated, and embedded in Epon-Araldite. Ultrathin sections stained with uranyl acetate and lead citrate were studied under a JEM-100B electron microscope. The results of quantitative measurements were processed statistically using Strelkov tables and the Fisher-Student method. Coefficients of linear and rank correlation were calculated routinely [10] on an ACBT-40-30 computer using designated software [8].

RESULTS

The data obtained (Table 1) show that vagotomy resulted in substantial structural reorganizations in the microvascular bed of the liver and in changes of the microcirculation which were most pronounced 5-14 days later. The reorganizations in the

TABLE 1. Change of Morphophysiological Parameters in the Microcirculatory System after Vagotomy

Time after vagotomy, days	Area of sinusoid	LCR	Kinetic parameter ($p2$)		pO_2
			phase I	phase II	
Control	2.51±0.11	151.2±10.2	1.79±0.29	2.38±0.17	27.3±2.8
5	6.33±0.09*	58.0±5.5*	2.71±0.74*	5.03±0.22*	11.7±0.4*
7	5.78±0.16*	73.6±6.4*	1.43±0.58	3.89±0.11*.	17.9±2.1*
14	5.45±0.20*	61.2±3.0*	2.13±0.47	6.25±0.39*	14.7±3.0*
30	4.34±0.15*	66.3±6.1*	2.50±0.43*	4.35±0.38*	12.8±2.9*
60	3.69±0.22	97.7±5.8	5.00±1.90*	7.14±0.51*	20.9±2.8
90	3.30±0.35	144.5±9.9	1.83±0.39	2.29±0.21	25.5±4.0

Note. LCR in ml/min/100 g tissue; pO_2: partial pressure of oxygen in hepatic parenchyma (mm Hg); $p2$: parameter of exponential function characterizing kinetics of uptake (phase I) and release (phase II) of hydrogen (dimensionless value). Asterisk denotes statistically reliable ($p<0.05$) differences between vagotomized and control rats.

microcirculatory system consist in a dilation of the sinusoidal capillaries and hemostasis in them, swelling of endotheliocytes and destruction of their organelles (mainly of mitochondria and cytoplasmic reticulum), an increase of the area of the perinuclear space, and widening of the pericapillary gaps, where fragments of destroyed hepatocytes and cells of the capillary lining are often found. The noted morphological changes correlate well with the LCR decrease, the kinetic modifications of the microcirculation, and with the pO_2 drop in the organ parenchyma. The magnitude of the morphological changes in the microvascular bed somewhat decreases 30 days postoperation, while the LCR, kinetic parameters of the microcirculation, and pO_2 remain at the previous level. The tendency for the LCR and pO_2 to drop persists against the background of near - normalization of the microvascular network structure 60 days after the operation and the changes in microcirculatory kinetics are also maintained. After 90 days none of the indexes of the microhemocirculatory system studied in the liver differ from the control.

In summary, it may be stated that the disruption of the vagal innervation of the liver is accompanied by disturbances of the microcirculation (LCR decrease and changes in kinetics), the morphological basis of which probably consists of a reorganization of the microhemovascular bed (widening of the sinusoidal capillaries and stasis) and of the ultrastructure of cells lining the capillaries (swelling and destruction of some endotheliocytes). The changes noted in morphophysiological parameters of the microhemocirculatory system correlate well with the pO_2 decrease in the parenchyma, which attests to a circulatory nature of the developing hypoxia. The pathogenic mechanisms of the latter may be discussed in terms of the following considerations. Vagotomy results in decentralization of the hepatic autonomic ganglia as well as neuronal dystrophy in some ganglionic cells, which induce changes in the transmitter background both in the ganglia themselves and in other structural components of the organ [4]. In addition, under such conditions the cells and tissues of the organ are deprived of trophic substances transported by the axoplasmic flow of vagus fibers. These factors may exert a direct or indirect (mediated by tissue basophil active substances) effect on the vascular smooth musculature, changing its tone, contractility, and reactive properties and thus leading to microcirculatory disorders and hypoxia [12,16]. Neurogenic mechanisms, such as a change of the excitation thresholds of visceral afferents under conditions of O_2 deficiency-induced distortion of the tissue chemism (acidosis and hypercapnia), may produce pathological reflexes [17], including those involving the regulatory microvessels. Another mechanism may be associated with the disturbed function of the higher nerve centers regulating the peripheral circulation under the influence of pathological impulses from the central ends of the cut vagus nerves. Furthermore, the increase of tissue O_2 uptake induced by epinephrine [7], the content of which in the organ is increased due to the predominance of the sympathetic tone [4], may maintain and aggravate the hypoxia under conditions of vagotomy. All the described mechanisms together induce and maintain the state of oxygen deficiency in the liver parenchyma, which triggers an entire cascade of secondary changes in metabolism and bioenergetics, resulting in damage to the biochemical, structural, and functional organization of the organ [3,5,15]. The hypoxic state in vagotomy persists for around a month and then gradually levels off, probably due to the development of compensatory and adaptive processes (the LCR increases and the reserve capillaries take over).

Thus, the disruption of the parasympathetic innervation of the liver results in the development of a hypoxic state in the organ, where microdynamic disorders play a key role.

REFERENCES

1. A. A. Afifi and S. P. Azen, *Statistical Analysis: A Computer-Oriented Approach* [in Russian], 2nd ed., Acad. Press (1979).
2. V. V. Berezovskii, in: *Polarographic Oxygen Determination in Biological Objects*, Kiev (1974), pp. 34-49.
3. Yu. A. Vladimirov and E. M. Kogan, in: *Biological Membranes and Aspects of Current Cardiology* [in Russian], Moscow (1979), pp. 10-11.
4. Yu. K. Eletskii, T. K. Dubovaya, and A. V. Bykov. in: *Cytological Mechanisms of Histogenesis* [in Russian], Tashkent (1983), pp. 58-60.
5. Yu. K. Eletskii, T. K. Dubovaya, and I. K. Mal'tseva, in: *Synopses of Reports of 4th Transcaucasian Conference of Morphologists* [in Russian], Tbilisi (1985), p. 69.
6. A. A. Zozulya, *Byull. Eksp. Biol. Med.*, **83**, № 4, 420-422 (1977).
7. V. I. Kulinskii, *Vopr. Med. Khim.*, № 6., 22-25, (1979).
8. *Collection of Scientific Software in FORTRAN* [in Russian], Vol. 1, Statistika, Moscow (1974).
9. Z. M. Sigal and A. S. Makarov, *Kazanskii Med. Zh.*, № 5, 374-380 (1983).
10. V. Yu. Urbakh, *Statistical Analysis in Biology and Medicine* [in Russian], Moscow (1975).
11. A. Yu. Tsibulevskii, M. D. Polivoda, and A. P. Ettinger, *Izv. Akad. Nauk SSSR, Ser. Biol.*, № 3, 461-465 (1989).
12. A. M. Chernukh, P. N. Aleksandrov, and O. V. Alekseev, *The Microcirculation* [in Russian], Moscow (1984).
13. K. Aukland, B. Bower,and R. Berliner, *Circulat. Res.*, **14**, 164-187 (1964).
14. H. Johanson and J. Lindhagen, *Metabolism*, № 12, 12-16 (1989).
15. D. Jones, *Progr. Clin. Biol. Res.*, **274**, 127-144 (1988).
16. A. Halaris, *Experientia*, **27**, 78-79 (1971).
17. G. Stahl, *Am. J. Physiol.*, **262**, № 3, Pt. 2, H748-H753 (1992).