11. W. F. Balistreri, Semin. Liver Dis., 2, 67 (1987).
12. H. David, P. Reinke, and M. Bimmler, Exp. Path., No. 4, 247 (1986).
13. W. Prinz, R. A. Schuhmann, and W. Kalbfleisch, Zbl. Gynäk., No. 5, 279 (1983).
14. E. Reinold, Obstet. Gynec., 32, 471 (1987).
15. B. Schultze, A. M. Kellerer, C. Grossman, et al., Cell Tissue Kinet., 3, 241 (1978).

HISTOCHEMICAL ANALYSIS OF ENZYMES OF TRANSPORT AND BIOENERGETICS OF RAT SMALL INTESTINAL ENTEROCYTES AFTER VAGOTOMY AND DIBUNOL ADMINISTRATION

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KEY WORDS: small intestine; enterocytes; enzymes; vagotomy; antioxidant

Activation of lipid peroxidation (LPO) in biological membranes plays a definite role in the pathogenesis of the neurodystrophic process developing in organs of the digestive system after vagotomy. The concentration of malonic dialdehyde, an end product of LPO, is increased in the liver of vagotomized rats [4]. It has also been shown that vagotomy leads to the development of hypoxia in the denervated organs (stomach, jejunum, liver) [13], and this is a powerful factor inducing LPO [10]. Meanwhile it has been demonstrated that vagotomy is accompanied by multiple disturbances, at varied depths, of the structure of cell membranes of the stomach, liver, pancreas, and intestine [3]. Disturbance of the compact and regular structure of the lipoprotein complexes of biomembranes leads as a rule to the activation of LPO [6]. The view that unphysiological intensification of LPO occurs in the digestive organs after vagotomy is supported indirectly by the fact that vitamin E has a positive effect on certain functional and morphological characteristics of hepatocyte mitochondria [5].

In this investigation an attempt was made, by pharmacological methods, to correct the postdenervation changes taking place in the epithelium of the small intestine, by the use of the phenolic antioxidant dibunul (2,6-di-tert-butyl-4-methylphenol). Activity of key enzymes of transport and bioenergetics, namely alkaline phosphatase (AlP) and succinate dehydrogenase (SDH), was chosen as the morphological criterion for evaluating the metabolic and functional state of the intestinal epithelial cells.

EXPERIMENTAL METHOD

Male albino rats (n = 53) weighing 180-210 g were used. Under anesthesia (150 mg Na γ-hydroxybutyrate + 0.6 mg diazepam (100 g body weight, respectively), bilateral subdiaphragmatic trunk vagotomy was performed on 30 rats and 15 rats served as the control. Starting with the 2nd day after the operation, half of the rats received dibunol intraperitoneally in a dose of 20 mg/kg, in a 3% solution of Tween-80. The rats were killed three at a time (intact, vagotomized, and vagotomized and receiving dibunol), 7, 14, and 30 days after vagotomy, and 6, 13, and 29 days, respectively, after the beginning of the course of injections. In a special series of experiments in order to estimate the effect of dibunol on the test parameters of the intact rats, the compound was injected in the same dose for 14 days. All the animals were deprived of food for 16-18 h before sacrifice. Fragments of the proximal part of the jejunum were frozen in liquid nitrogen and mounted in blocks. In the frozen sections AlP was detected by the method in [1] and SHD by the method in [8]. Photometry was carried out with the MTsFU-2MP scanning microscope-photometer at a wavelength of 546 nm. At least 15 scanning fields per section were tested photometrically (9 measuring points in each field); the size of the fields corresponded to the brush border (in the case of AlP) and the apical pole (SDH) of the enterocytes. The choice of cells for photometry was made randomly within the epithelium of the intestinal villi. The results of the measurements were subjected to statistical analysis with the aid of Strelkov's tables [11].

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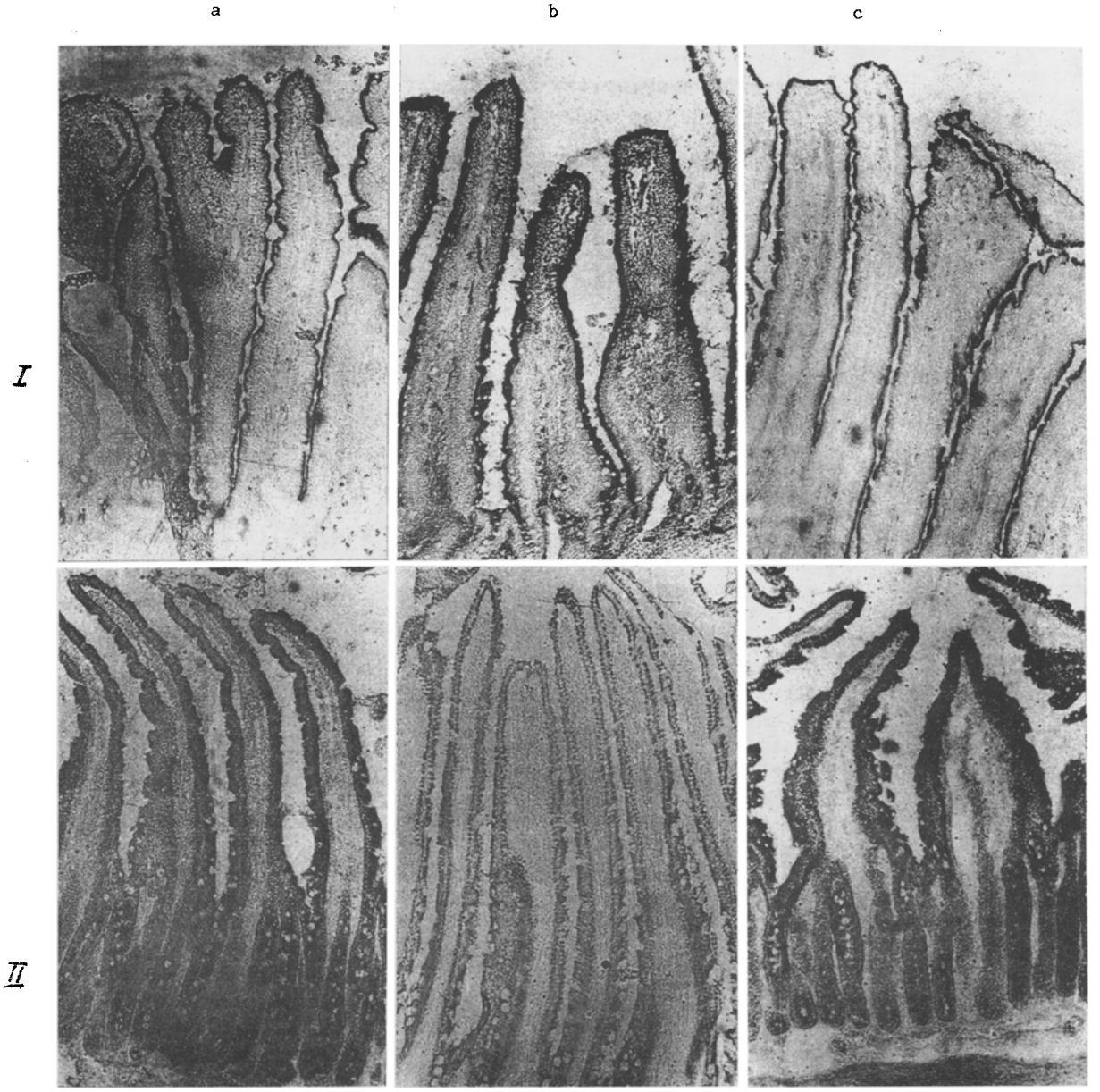

Fig. 1. Changes in AlP (1 and 7 days) and SDH (11 and 30 days) activity in intestinal epithelial cells after vagotomy and injection of dibunol. a) Intact rats; b) vatotomized; c) vagotomized and receiving dibunol.

EXPERIMENTAL RESULTS

Histochemical analysis showed that AlP activity in epithelial enterocytes of the intestinal villi of intact rats was observed in the brush border region, SDH mainly in the apical pole of the cells. The SDH was present as tiny, discrete particles, corresponding in all probability to mitochondria, for this enzyme is known to be firstly bound with the inner mitochrondrial membrane [7]. Control experiments showed that injection of dibunol into intact rats for 2 weeks causes no significant changes in the activity or topography of these two enzymes. Although vagotomy did not change the location of AlP, it led to an increase in its activity 7 days after the operation and to a decrease 30 days after it. The AlP activity was unchanged after 14 days. This parameter in vagotomized rats receiving dibunol did not differ significantly from its value in intact animals. Vagotomy was followed by a decrease in SDH activity after 7 and 30 days (Fig. 1); after 14 days there was only a tendency for its activity to decrease ($p \approx 0.05$). The intracellular topography of SDH was unchanged under these circumstances. In the group of vagotomized rats receiving dibunol, SDH activity was close to the corresponding value in the control animals (after 14 and 30 days: see Table 1).

TABLE 1. Changes in AlP and SDH Activity of Enterocytes of Intestinal Villi after Vagotomy and Injection of Dibunol

Experimental conditions	Enzyme	7 days	14 days	30 days
Intact	AIP	100±1,0	100±7,0	100±2,2
	SDH	100±1,6	100±6,7	100±1,9
Vagotomy	AIP	114,0±1,8*	102,8±5,4	85,5±1,7*
	SDH	89,2±2,1*	91,9±2,9	87,3±2,3*
Vagotomy + dibunol	AIP	95,6±3,1**	102,0±4,8	98,0±1,8**
	SDH	90,6±1,5	93,5±4,3	104,8±2,5**

Note. *) Statistically significant differences between intact and vagotomized rats; **) between vagotomized rats receiving and not receiving dibunol.

It can be concluded from these observations that vagotomy leads to regular changes in the pattern of enzyme activity of enterocytes in the jejunal villi, which follow a wave-like course in time and are tantamount to an increase in AlP activity in the region of the brush border after 7 days and a decrease after 30 days, and a decrease in SDH activity in the apical pole of the cells after 7 and 30 days. Thus the dynamics of the changes in the enzyme profile of the enterocytes after vagotomy is in agreement with the general kinetics of the morphological and functional transformations observed in the small intestine under these conditions: the greatest changes are found on the 7th and 30th days, less marked changes on the 14th day [12]. The increase in AlP activity in the zone of the brush border of the enterocytes of the intestinal villi 7 days after vagatomy evidently reflects increased permeability of the intestinal barrier in the early period of the postvagotomy syndrome [16]. The decrease in AlP activity after 30 days correlates with the decrease in SDH activity, and may probably be evidence of inhibition of metabolic and bioenergetic processes in the enterocytes at this period after vagotomy. As regards the causes of the decrease in activity of these enzymes under these conditions, it can be tentatively suggested that it is connected with disturbance of the balance between neurotransmitters (acetylcholine and catecholamines) in the tissues of the organ, for that may have a significant effect on both biosynthesis and activity of enzymes [2, 9]. Another factor leading to inhibition of AlP and SDH after a vagotomy may be activation of LPO, for certain LPO products (free radicals, hydroperoxides, etc.) are known to inhibit enzymes, especially those which are membrane-dependent [14, 15]. Support for this hypothesis may be provided by the normalizing influence of dibunol on changes in ALP and SDH activity caused by vagotomy, which was demonstrated in the present study. This circumstance raises the question of whether antioxidants can be used for the pharmacological correction of small-intestinal complications arising in patients with duodenal ulcer undergoing surgical operations accompanied by vagotomy.

LITERATURE CITED

1. M. Burstone, Enzyme Histochemistry [Russian translation], Moscow (1965).
2. S. N. Golikov, V. B. Dolgo-Saburov, N. R. Elaev, and V. I. Kuleshov, Cholinergic Regulation of the Biochemical Systems of the Cell [in Russian], Moscow (1985).
3. Yu. K. Eletskii, Morphological and Functional Analysis of Organs of the Digestive System after Disturbance of Their Innervation [in Russian], Moscow (1984), pp. 6-16.
4. Yu. K. Eletskii, T. K. Dubovaya, and I. K. Mal'tseva, Abstracts of Proceedings of the 4th All-Union Conference on Cell Pathology [in Russian], Moscow (1987), p. 161.
5. A. A. Zozulya and Yu. K. Eletskii, Farmakol. Toksikol., 40, No. 1, 70 (1977).
6. S. V. Konev, S. L. Aksentsev, G. D. Nisenbaum, and I. M. Okun', Free-Radical States and Their Role in Radiation Sickness and Malignant Growth [in Russian], Moscow (1971), pp. 48-49.
7. V. N. Luzikov, Regulation of Formation of Mitochondria: Molecular Aspects [in Russian], Moscow (1980).
8. A. G. E. Pearse, Histochemistry [Russian translation], Moscow (1963).
9. N. I. Razumovskaya, Biokhimiya, 36, No. 4, 702 (1971).
10. V. I. Sorokovoi, Yu. M. Petrenko, and N. N. Mochenova, Byull. Eksp. Biol. Med., 98, No. 11, 543 (1984).

11. R. B. Strelkov, Statistical Tables for Express Analysis of Experimental and Clinical Data [in Russian], Obninsk (1980).
12. A. Yu. Tsibulevskii, Morphological and Functional Analysis of Organs of the Digestive System after Disturbance of Their Innervation [in Russian], Moscow (1984), pp. 80-88.
13. A. Yu. Tsibulevskii, 14th All-Union Conference on the Physiology of Digestion and Absorption [in Russian], Ternopol'-L'vov (1986), pp. 306-307.
14. Y. Kono and J. Fridovich, J. Biol. Chem., 257, No. 10, 5751 (1982).
15. N. Shirumi, K. Kobayashi, and K. Hayashi, J. Biol. Chem., 259, No. 7, 4414 (1984).
16. J. Rabinowitsch, Am. J. Physiol., 82, 279 (1927).

PINEAL GLAND AND THE APUD SYSTEM

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KEY WORDS: pineal gland, APUD system

Most research workers include the secretory cells of the pineal gland, or pinealocytes, in the diffuse neuroendocrine system (the APUD system) [2-4, 9]. However, the ultrastructure of the pinealocytes and of the pineal gland as a whole in the adult human has not yet been studied. There have been only solitary electron-microscopic studies, conducted on the human embryonic pineal gland (PG) [11, 12, 14].

The aim of this investigation was a comparative study of cells of the APUD system and adult human and guinea pig pinealocytes.

EXPERIMENTAL METHOD

Altogether 10 PG from persons dying from various cardiovascular diseases (age 40-66 years; five men and five women) and 20 PG from female guinea pigs were studied. Autopsy was carried out 2.5 h after death. The animals (four equal groups) were killed by decapitation under ether anesthesia, at 6 a.m., noon, 6 p.m., and midnight in the course of the 24-h period. The traditional methods of light and electron microscopy were used, with the IEM-100B electron microscope. Stereometric ultrastructural analysis of the guinea pig pinealocytes was carried out on electron micrographs with magnification of 18,000, using a test system with 513 nodal points and a grid with a step of 0.5 cm [1].

EXPERIMENTAL RESULTS

The human and guinea pig PG has an organ-like structure, and in its histoultrastructural parameters it is a neuroendocrine gland, separated from surrounding brain structures by a thin connective-tissue capsule (Fig. 1a). Properties of nerve and endocrine tissues are combined in PG: its neurosecretory cells are closely interconnected with the glial component, blood vessels, and nerve endings (Fig. 1b).

Pinealocytes account for the majority of all cells of the pineal parenchyma (79.9%), whereas gliocytes account for only 15.1%. In other words, in PG there is a kind of quantitative deficit of the glial component compared with brain tissue [5]. Unlike cells of the APUD system, pinealocytes have a neuron-like structure. A perikaryon or trophic center and numerous cytoplasmic processes can be distinguished in their cytoplasm. The cell nuclei, measuring 6.82 ± 0.2 μm, contain delicate chromatin, arranged mainly near the inner membrane of the karyolemma. The nuclear membrane has three or four nuclear pores, through which the ribonucleotide enters the perikaryon. The processes are densely interwoven with each other and they take part in desmosome-like and gap junctions with the neighboring pinealocytes and glial cells (Fig. 2a).

The presence of secretory vesicles with transparent contents or with an electron-dense core in the perikaryon and cytoplasmic processes is morphological evidence of the endocrine

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