EFFECT OF TISSUE-SPECIFIC INHIBITORS OF PROLIFERATION ON MITOTIC ACTIVITY AND ADHESION OF LIVER CELLS WITH DISTURBED INNERVATION

Yu. K. Eletskii, V. B. Zakharov,
K. G. Biryukov, E. A. Modyanova,
L. I. Krivov, S. G. Mamontov,
and T. K. Dubovaya

UDC 616.36-018.1-02:616.833.19-089.85]-092-07

KEY WORDS: liver parenchyma; innervation; chalones; regeneration.

The mechanisms controlling cell proliferation have not yet been adequately studied. One particular aspect of this problem is that of interconnection between different levels of regulation of the intensity of cell division and, in particular, at the whole body and tissue levels. The first of these is represented by integrating systems, the second by chalones. There is reason to identify with chalones certain special substances known as adhesins, which participate in the mechanical integration of the cellular components of tissues [6, 9]. These ideas provide a basis for understanding the mutual dependence of the degree of "coupling" of cells and their proliferative activity. Integrating systems are known to have a mainly nonspecific influence on cell division whereas chalones inhibit cell multiplication in those tissues in which they are formed, i.e., they exhibit marked tissue specificity. Accordingly it is particularly interesting to study the biological activity of specific regulators of proliferation when the activity of the integrating systems and, in particular, the innervation of the organ, is disturbed.

The aim of this investigation was to study, first, certain cytophysiological features of the liver parenchyma when its innervation is disturbed, and second, the effectiveness of action of chalones on the level of mitotic activity and the degree of adhesion of the hepatocytes when the parasympathetic and sympathetic innervation of the normal and regenerating liver is disturbed.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 160-180 g were used. The animals were kept on a fixed program of 12 h of daylight (6 a.m. to 6 p.m.) and 12 h of darkness (6 p.m. to 6 a.m.) and were allowed food ad lib. The parasympathetic innervation was disturbed by bilateral subdiaphragmatic vagotomy, performed 1, 2, 3, and 4 weeks before the experiment. The sympathetic innervation was disturbed in another group of animals by injection of guanethidine in a dose of 10 mg/100 g body weight daily for 3 weeks, starting with the first day after birth. Two-thirds of the liver was removed from some animals, which were killed 24 h after the operation. The procedure of isolation of chalones from the rat liver was described previously [5]. The chalone-containing preparation was injected in a dose of 50 mg per rat once or 3 times at intervals of 8 h; investigations were carried out 4 h after the last injection of the preparation. The intensity of cell multiplication was estimated by counting MI_{col} in films of hepatocytes dissociated with alkali. Colchicine in a dose of 1 mg/kg was injected 3 h before sacrifice. The strength of adhesion was determined by Coman's method in the modification in [7]. Alanine aminotransferase (ALT) activity in the blood serum was determined as the criterion of permeability of the hepatocyte plasmalemma.

EXPERIMENTAL RESULTS

If the innervation of the liver was intact, hepatic chalones had virtually no effect on the initially low level of proliferation in the intact liver, but the force of adhesion of

Department of Histology and Embryology, Faculty of Internal Medicine, and Laboratory of Cell Biology and Laboratory of Experimental Biological Models, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 2, pp. 239-242, February, 1989. Original article submitted June 20, 1988.

0007-4888/89/0002-0259$12.50

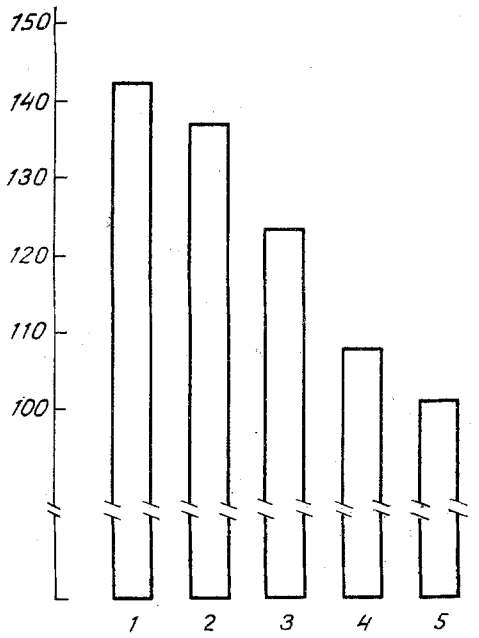

Fig. 1

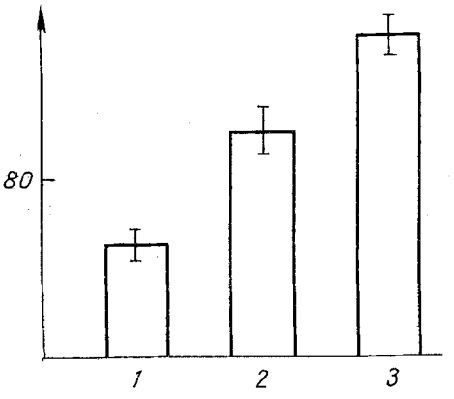

Fig. 2

Fig. 1. Strength of coupling of hepatocytes at different times after bilateral subdiaphragmatic vagotomy. Ordinate, strength of coupling of hepatocytes (in μg per cell); abscissa: 1) intact liver, 2) 1 week after vagotomy, 3) 2 weeks after vagotomy, 4) 3 weeks after vagotomy, 5) 4 weeks after vagotomy.

Fig. 2. Serum ALT activity. Ordinate: ALT activity (in U/liter); abscissa, series of experiments: 1) control, 2) 2 weeks after vagotomy, 3) 1 month after vagotomy.

the hepatocytes was significantly increased (Table 1). A similar increase in the "coupling force" of the cells in the regenerating liver was accompanied by a sharp decrease in mitotic activity. It was noted that 24 h after partial hepatectomy, i.e., at the height of proliferative activity of the hepatocytes, parameters of the force of cell coupling were the same as in the control. The impression is obtained that this fact is evidence against the notion that a causative connection exists between the degree of adhesion of the cells and the level of proliferation. However, resting hepatocytes are known to commence the cycle a few hours after partial hepatectomy. It is at this trigger moment that adhesion of hepatocytes is sharply reduced [8].

After vagotomy there was a gradual decrease in the strength of coupling of the hepatocytes in the animals' liver (Fig. 1) and an increase in their serum ALT activity (Fig. 2), indicating a disturbance of the state of the plasmalemma and an increase in its permeability. Both parameters showed a tendency to rise during the period of investigation. After disturbance of the vagal innervation, the ultrastructure and function of the hepatic parenchyma changed regularly [3]. The greatest destructive changes in this case developed during the first weeks, and by the end of a month, a compensatory reaction was clearly evident. If values of the strength of adhesion of the hepatocytes are compared with data on the structure and function of the hepatic parenchyma, it will be clear that the intensity of coupling of the parenchymatous cells was significantly below normal both at the height of the destructive phenomena (1.2 weeks after denervation) and on the appearance of compensatory processes (after 1 month). Particular attention is drawn to correlation between parameters of the state of the plasmalemma (serum ALT activity) and the strength of adhesion of the hepatocytes, for the properties of the cell memebrane are known to largely determine the character of intercellular relations and, in particular, the state of the intercellular junctions.

Mitotic activity of the hepatocytes was increased 1 month after vagotomy (Table 1). This fact can evidently be regarded as a component of compensatory and adaptive processes.

Injection of the chalone-containing extract from the liver into vagotomized rats led to an increase in the strength of coupling of the cells, up to the control value. However, no

TABLE 1. Mitotic Activity (MA) and Strength of Adhesion of Hepatocytes when Innervation of the Liver is Disturbed and Chalones Administered

	MI_{col}, ‰	Strength of adhesion, μg per cell
Control (n = 9)	0,02±0,01	143±6
Hepatic chalones, single injection (n = 6)	0,01±0,006	229±2*
Hepatic chalones, repeated injections (n = 6)	0,02±0,005	219±2*
Hepatectomy (24 h; n = 5)	20,70±4,70*	131±4
Hepatectomy + hepatic chalones, single injection (n = 6)	6,77±0,47**	224±5**
Hepatectomy + hepatic chalones, repeated injection (n = 5)	2,96±0,90*, **	227±3*,
Vagotomy, 4 weeks (n = 6)	3,64±0,81*	123±5*
Vagotomy, 4 weeks + hepatic chalones single injection (n = 6)	3,67±0,38*	158±10*, ***
Desympathization (n = 6)	2,52±0,58*	214±7*
Desympathization + hepatic chalones, single injection (n = 5)	1,55±0,17	224±4
Desympathization + hepatic chalones, repeated injections (n = 6)	1,39±0,39	267±22*4

Legend. *) Values of MI and strength of coupling of cells differ from control values (group 1): $0.001 < p < 0.01$. **) Values differing significantly from corresponding values for hepatectomized animals (group 4): $0.001 < p < 0.02$. ***) Values of strength of adhesion of hepatocytes differ significantly from this parameter in vagotomized rats (group 7): $p = 0.01$. *4) Values of strength of coupling of hepatocytes differ significantly from this parameter after desympathization (group 9): $p < 0.05$.

significant change in mitotic activity took place. Consequently, hepatic chalones, in the dose used, while potentiating the adhesive properties of hepatocytes, had no effect, meanwhile, on proliferative processes in the tissue as a whole. The regenerating liver, with disturbance of its parasympathetic innervation, likewise proved insensitive to tissue inhibitors of proliferation [4].

The strength of coupling of the hepatocytes in desympathized rats changed in the opposite manner to that in vagotomized animals, namely it was considerably reduced. It is interesting to note that mitotic activity also increased. After total sympathectomy, achieved by injecting antibodies to nerve growth factor into the animals, Volkova and Tarabrin [1, 2] observed intensification of proliferation. Injection of chalones into sympathectomized rats in different experiments reduced mitotic activity by 40-45%.

Consequently, preservation of the parasympathetic innervation is the essential and sufficient factor for manifestation of the action of tissue inhibitors of cell division (chalones) on adhesion and on the intensity of cell proliferation. Meanwhile its disturbance, i.e., predominance of the sympathetic influence on the tissue, leads to disappearance or a sharp decline of the sensitivity of hepatocytes to the inhibitory effect of chalones on cell division.

The character and degree of the proliferative response of a denervated tissue to tissue regulators of cell proliferation are thus determined by the quality of the residual neurotrophic influence: parasympathetic or sympathetic. It can accordingly be postulated that the trophic influence of the two divisions of the autonomic nervous system is immediated (realized) by different substances.

LITERATURE CITED

1. O. V. Volkova and S. B. Tarabrin, Arkh. Anat., No. 7, 5 (1975).
2. O. V. Volkova, The Neurodystrophic Process (Technical Aspects) [in Russian], Moscow (1978).
3. Yu. K. Eletskii, T. K. Dubovaya, A. V. Bykov, et al., Cytologic Mechanisms of Histogenesis [in Russian], Tashkent (1983), pp. 58-60.
4. Yu. K. Eletskii, S. G. Mamontov, T. V. Savchenko, and T. K. Dubovaya, Byull. Éksp. Biol. Med., No. 10, 482 (1984).
5. V. B. Zakharov, Byull. Éksp. Biol. Med., No. 5, 584 (1980).
6. A. G. Malenkov and G. A. Chuich, Intercellular Junctions and Tissue Reactions [in Russian], Moscow (1979).
7. E. A. Modyanova, O. A. Bocharova, and V. F. Ushakov, Byull. Éksp. Biol. Med., No. 4, 459 (1980).
8. L. A. Radkevich, Biofizika, 24, No. 5, 906 (1981).
9. V. P. Yamskova, Chalones: Their Importance and Role in Normal and Pathologial Processes [in Russian], Moscow (1981), pp. 60-67.

CYTOCHEMICAL STUDY OF LOCUS COERULEUS NEURONS AFTER GUANETHIDINE DESYMPATHIZATION IN RATS

G. Altan, V. N. Yarygin, and A. V. Grigor'eva

UDC 612.827.014.22].014.46.09]-08

KEY WORDS: locus coeruleus; desympathization; histones; template activity; noradrenalin fluorescence.

Convincing evidence has been obtained in the last decade that the principal noradrenergic brain formation, the locus coeruleus (LC), is involved in the regulation of the most important autonomic functions of the body (vascular tone, temperature regulation, motor activity, etc.) [11, 13].

From the structural point of view LC can evidently be regarded as a link in the chain of visceral afferent pathways maintaining the autonomic component of several important physiological acts [10], and also as an essential component of afferent synthesis in the homeostasis maintaining system [1].

Great similarity is observed between the noradrenergic system of the brain and the sympathetic division of the peripheral nervous system with respect to several morphological, biochemical, and physiological parameters. It has even been suggested that LC is the "cranial ganglion" of the sympathetic system [8].

One possible experimental approach to the problem of relations between peripheral and central noradrenergic structures is to study the response of the central component to structural and functional changes in the peripheral component. In particular, by recourse to a model of measured desympathization, it is possible to monitor the morphological and functional state of LC neurons under conditions of death of a certain number of sympathetic neurons, since guanethidine has no direct effect on brain cells [9].

The aim of this investigation was to study transcription and the histone component of chromatin, and also to undertake a quantitative microfluorometric estimation of the noradrenalin (NA) concentration in the cytoplasm of LC neurons of intact and partially desympathized adult rats.

N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR O. S. Andrianov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 2, pp. 242-245, February, 18989. Original article submitted March 5, 1988.

 0007-4888/89/0002-0262$12.50