DNA CONTENT IN INTRACARDIAL NEURONS OF INTACT AND DESYMPATHIZED RATS

A. V. Grigor'eva and V. N. Yarygin

UDC 612.178:612.398.145.1

The DNA content in the nuclei of the intracardial neurons of intact rats and of rats with a sharply reduced number of neurons in the sympathetic ganglia following injection of guanethidine during the first 14 days after birth, was investigated cytospectrophotometrically. The study of the DNA content in the nuclei of these neurons in intact animals during early postnatal ontogeny revealed the appearance of a certain percentage of cells with an increased DNA content in the nuclei in rats weighing 100-300 g. A marked increase in the percentage of cells with an increased DNA content was observed in desympathized animals weighing 300 g. The observed increase in the DNA content in the nuclei of the intracardial neurons during postnatal ontogeny in rats under normal conditions and after desympathization may be conjecturally attributed to the appearance of intracardial cholinergic structures.

KEY WORDS: intracardial neurons; DNA; desympathization.

The cytophotometric study of the DNA content in neurons has revealed a considerable distribution of polyploid neurons in various parts of the CNS.

The autonomic nervous system has received very little study in this respect. However, the presence of polyploid forms has been demonstrated among the neuron population of the superior cervical ganglion of rabbits [10] and the intramural ganglia of the mouse heart [6].

Investigations of the cells in various parts of the nervous system, as well as in other highly differentiated tissues, have shown that polyploidization takes place regularly in a certain period of postnatal ontogeny [2, 4, 18]. Some workers emphasize the fact that a change in the conditions of ontogeny can exert a significant effect on the conversion of the cells into the polyploid state [2].

The DNA content in neurons of the intramural ganglia of the heart was studied in intact rats at various stages of postnatal ontogeny and in rats with a sharply reduced number of neurons in the ganglia of the lateral sympathetic chain as the result of chemical desympathization.

EXPERIMENTAL METHOD

Noninbred albino rats were used. Material was taken from intact newborn rats and rats weighing 50, 100, 200, and 300 g and from desympathized animals weighing 300 g.

Partial desympathization was carried out by daily subcutaneous injections of guanethidine (15 mg/kg) into newborn rats for the first 14 days after birth [14, 16]. The degree of desympathization was determined by calculating the ratio between the number of cells in the stellate ganglion of the desympathized animals and their number in the intact control.

Department of Morphology and Cytology, Medical Biological Faculty, and Central Research Laboratory, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopokhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 10, pp. 104-107, October, 1974. Original article submitted December 17, 1973.

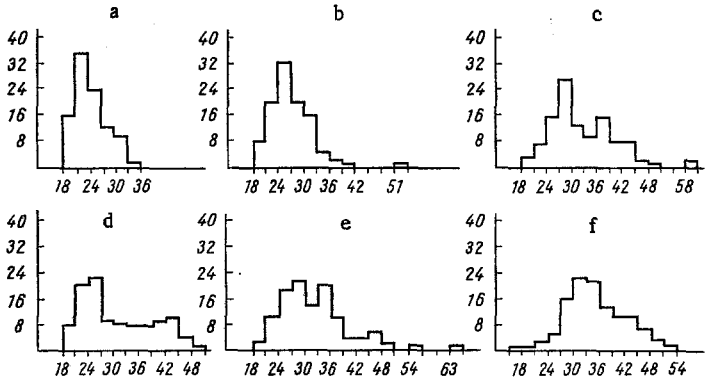

Fig. 1. DNA content in nuclei of intracardial neurons of intact and desympathized rats (combined histograms for 3 animals): a) newborn intact rats; intact rats weighing b) 50 g, c) 100 g, d) 200 g, e) 300 g; f) desympathized rats weighing 300 g (150 cells, level of desympathization not more than 30%). Abscissa, DNA content (in conventional units); ordinate, percentage of nuclei with a given DNA content compared with the total number of nuclei investigated for that age group.

DNA was determined quantitatively in the nuclei by a cytospectrophotometric method using the "Shimadsu" universal spectrophotometer at a wavelength of 540 nm. Sections 20 μ in thickness, stained by Feulgen's method, were used for photometry. Sections of this thickness were chosen because it is greater than the largest diameter of the nuclei of the neurons to be investigated, so that only those nuclei that lay practically entirely in the thickness of a given section could be examined photometrically. The error connected with photometry of sections was thereby substantially reduced [17].

The DNA content in conventional units was calculated as the product of the optical density of the nucleus and the area of its greatest cross-section. The area was determined as the product of the maximal and minimal radii of a cross-sectionof the nucleus drawn by means of a drawing apparatus.

The χ^2 criterion was used to assess the statistical significance of the difference between the histograms obtained [5].

EXPERIMENTAL RESULTS

The results of measurement of the DNA content in the nuclei of neurons of the intramural ganglia of intact animals are given in Fig. 1a-e. Comparison of the histograms shows that in rats weighing 100 g a certain percentage of cells with an increased DNA content in their nuclei compared with cells of the newborn animals appears for the first time. The difference was statistically significant ($P < 0.01$). Cells of this type were found also in 2 of the 3 animals investigated weighing 100 and 300 g.

The results of investigation of the nuclei of nerve cells of rats weighing 300 g, receiving guanethidine injections during the first 14 days after birth, are given in Fig. 1f. Comparison of these histograms with those of intact animals of the same weight (Fig. 1e) indicates a significant increase in the percentage of cells with a high DNA content in the nuclei in the desympathized rats ($P < 0.01$).

The results indicate that polyploid forms can be found among neurons of the intramural zone of the autonomic nervous system, as well as among neurons of the sympathetic ganglia and certain parts of the DNA.

On the basis of the results of a comparison of certain parameters of diploid and polyploid somatic cells it has been postulated that cells with an increased DNA content have a higher functional potential [2, 3]. It has also been shown that an increase in the DNA content in the nuclei of neurons during the ontogeny of certain parts of the nervous system coincides with the period of most rapid growth of the axons and dendrites and with the acquisition of morphological features characteristic of the cells of adult animals by the perikarya [15, 19].

With this in mind an attempt was made to compare the results of the present experiments with data in the literature on the formation of the nervous regulation of the heart during postnatal ontogeny.

Investigations have shown that in mammals there is a shift with age from sympathetic-adrenergic mechanisms of regulation of cardiac activity to vagal-cholinergic mechanisms [1, 11]. The absence of vagal influences on the heart in animals immediately after birth is connected by these workers with the absence of vagal tone at that age and with the immaturity of the peripheral nervous system [9]. In fact, morphological investigations of the cell composition of the intramural ganglia of the heart in postnatal ontogeny have shown that in newborn mammals they consist largely of almost and completely undifferentiated cells. Not until the age of 1.5 months do most neurons of the intracardial ganglia come to resemble the cells of adult animals. The final formation of the microstructure of these cells is completed by the time of sexual maturity [7, 12]. Krokhina [9], who studied the level of cholinesterase activity in neuron bodies and plexuses of the intramural apparatus of the heart, showed that the constantly high level of cholinesterase activity characteristic of the cells of adult animals is not established until the age of 1.5-2 months.

The increase in the DNA content in the intracardial neurons of rats, as the present investigation showed, takes place at about 1.5 months, i.e., at the same time as the structural changes described above. This coincidence suggests that the observed increase in the DNA content in the neurons of the intramural ganglia of the heart during ontogeny of normal rats may be connected with the formation of intracardial nervous structures participating in the cholinergic mechanisms controlling cardiac activity.

Considering the views expressed above on the character of formation of the nervous regulation of cardiac activity in ontogeny, it may be expected that desympathization of newborn animals would create abnormal conditions for the formation of the vagal-cholinergic component of the nervous apparatus of the heart.

According to data given by Olson and Malmfors [20], desympathization leads to increased proliferation of fibers of parasympathetic nature in the denervated region. Volkova et al. [6], when assessing the morphological reaction of intramural neurons of the mouse heart to destruction of the sympathetic cells by antibodies against nerve growth factor, observed that the nerve cells they studied were hypertrophied. With these observations in mind it can be expected that processes of hypertrophy of the intracardial cholinergic structures in early postnatal ontogeny taking place under normal conditions (and, according to the results of the present experiments, coinciding with an increase in the DNA content in some intracardial neurons) should be intensified after desympathization. In that case the increase in the number of nerve cells with an increased DNA content in the intracardial ganglia observed in the desympathized rats in the present experiments could be conjecturally associated with the intensification of growth of the cholinergic components of the intracardial ganglia under the conditions observed.

An alternative hypothesis would be that the change in the DNA content in the experiments described concerned the adrenergic nerve cells, the presence of which in the intracardial ganglia has been demonstrated in recent years by a number of workers [8, 13]. It will be difficult, however, to expect that the number of neurons of this type in the heart could be large and, for that reason, this alternative seems unlikely.

LITERATURE CITED

1. I. A. Arshavskii, in: Self-regulation of the Heart [in Russian], Moscow (1967), p. 13.
2. V. Ya. Brodskii, Cell Nutrition [in Russian], Moscow (1966).
3. V. Ya. Brodskii and I. V. Uryvaeva, Ontogenez, No. 3, 229 (1970).
4. V. Ya. Brodskii, G. A. Sokolova, and T. E. Manakova, Ontogenez, No. 1, 33 (1971).
5. B. L. Van der Waerden, Mathematical Statistics, Springer-Verlag (1969).
6. O. V. Volkova, V. Ya. Brodskii, and M. Z. Chunaeva, Byull. Éksperim. Biol. i Med., No. 5, 97 (1973).
7. N. A. Kiseleva, Zdravookhr. Kazakhstana, No. 4, 20 (1966).
8. E. M. Krokhina and E. K. Plechkova, Dokl. Akad. Nauk SSSR, 196, No. 1, 211 (1971).
9. E. M. Krokhina, Vestn. Akad. Med. Nauk SSSR, No. 1, 72 (1968).
10. A. A. Kushch and V. N. Yarygin, Tsitologiya, No. 7, 228 (1965).
11. B. S. Musin, in: Abstracts of Proceedings of the 14th Conference of Junior Scientific Workers at the Institute of Normal and Pathological Physiology [in Russian], Moscow (1968), p. 49.
12. G. A. Pobedonostseva, Trudy Saratov. Med. Inst., 56, 73 (1968).
13. V. I. Skok, The Physiology of Autonomic Ganglia [in Russian], Leningrad (1970).
14. P. U. Angeletti and R. Levi-Montalchini, Proc. Nat. Acad. Sci. (Washington), 69, 186 (1972);
15. M. P. Del Cerro and R. S. Snider, J. Comp. Neurol., 133, 341 (1968).
16. J. Jensen-Holm and P. Juul, Acta Pharmacol. (Copenhagen), 30, 308 (1971).

17. L. W. Lapham, Science, 159, 310 (1968).
18. R. D. Lentz and L. W. Lapham, J. Neuropath. Exp. Neural., 29, 43 (1970).
19. J. L. O'Leary, J. Inukar, and J. M. Smith, J. Comp. Neurol., 142, 377 (1971).
20. L. Olson and T. Malmfors, Acta Physiol. Scand., 348, Suppl. 1 (1970).