ACETYLCHOLINESTERASE ACTIVITY IN CAUDAL PARTS OF THE MEDULLA

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The localization of acetylcholinesterase (ACE) activity was determined histochemically in some nuclei of the caudal part of the medulla (parvocellular, ventral, and gigantocellular reticular nuclei, vestibular nuclei, and the region of the tractus solitarius). Topographic schemes of the localization of ACE activity in the various morphological structures of the medulla were drawn up. Schemes of localization of cells possessing cholinesterase activity were prepared from the results of microscopic investigation of the reticular nuclei.

The results of experiments undertaken to study the neurochemical organization of the reticular formation of the medulla and pons suggests that the principal mediator in this part of the brain is acetylcholine [1, 7, 11, 12, 14]. However, cholinesterase activity is low in the medulla [3, 5, 8].

The results of histochemical investigations are conflicting. Some workers [13] report very low acetylcholinesterase (ACE) activity in the medullary reticular formation, while according to others [4, 6, 10], the nuclei of the reticular formation and several other structures of the medulla possess marked cholinesterase activity. Moreover, the primary purpose of these histochemical studies was to determine the general pattern of localization of the enzyme without determination of its activity at different levels of the individual medullary structures.

The object of the present investigation was to study ACE activity in various medullary structures and to prepare topographic schemes of localization of the enzyme detected by a histochemical method.

EXPERIMENTAL METHOD

Gerebtsoff's method [9] was used to detect active ACE and butyrylcholinesterase (BCE). Sections were cut to a thickness of 15 μ on a freezing microtome. The plane of section was perpendicular to the surface of the rhomboid fossa. The localization of the detected ACE was studied at different levels of the medulla in accordance with schemes of transverse sections of the cat medulla [2]. The incubation time (1 h) and the conditions of processing and staining the material were strictly identical in all experiments.

After the histochemical reaction the sections were subjected to histophotometry. So that the distribution of particles of absorbent (in this case, cells 10-100 μ in diameter) could be taken to be sufficiently uniform, comparatively large fields were examined photometrically.

The type MF-4 microphotometer was used for this purpose, and its operating conditions were chosen so that the area of the field examined was $100 \times 200\ \mu^2$. Photometry was carried out consecutively in a longitudinal direction, so that about 2500 fields were examined in each section.

The preliminary assessment of the relative intensity of staining of the field obtained by photometric examination was made in conventional units (c.u.). Since the intensity of staining of the field was determined relative to the least stained part of the section examined, the intensity of staining of this part was

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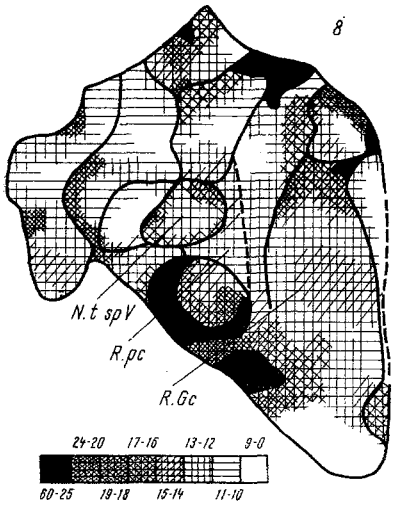

Fig. 1

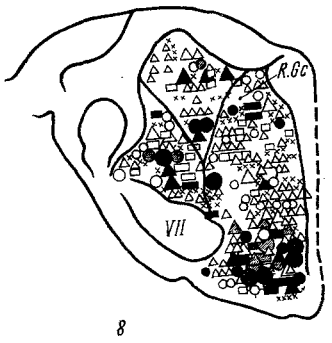

Fig. 2

Fig. 1. Scheme of quantiative distribution of ACE activity in the medulla at the level of the middle third of the facial nerve nucleus (similar schemes were prepared for all sections investigated). Conventional units were used for the scale of intensity. 8) Serial No. of scheme of transverse section through medulla at level of middle third of facial nerve nucleus in accordance with Grantyn's atlas of the cat's medulla and pons [2]. N.t.sp.V) Nucleus of spinal tract of trigeminal nerve; R.Gc) gigantocellular reticular nucleus; R.pc) parvocellular reticular nucleus; VII)facial nerve nucleus.

Fig. 2. Scheme of localization of reticular cells containing different amounts of ACE at the same level of the medulla as in Fig. 1 (similar schemes were prepared for all sections examined). Triangular cells are represented by triangles, oval and fusiform cells by circles, trapezoid cells by rectangles, and multipolar cells by figured circles. Different sizes of the conventional symbols correspond to different sizes of the cells: from 10 to 20, from 21 to 30, from 31 to 40, and over 41 μ. Black figures represent cells with well defined outlines and intensively stained cytoplasm; shaded figures represent cells in which ACE is detected at the cell border and in the cytoplasm, but the intensity of staining is only slightly different from the background of the section; unshaded figures represent cells in which ACE is determined only at the cell border; x denotes fibers revealed by staining for ACE. Conventions of representation of reticular nuclei are the same as in Fig. 1.

taken as the zero level in each section. In this way the data were made suitable for statistical analysis, thereby reducing the error of the results and, in particular, virtually eliminating any inaccuracy of quantitative photometric assessment due to slight differences between the zero levels in the sections.

A parallel microscopic study was made of the sections (ocular micrometer 15×, objective 40×) in which ACE had been revealed, and also of sections stained with toluidine blue. The number of cells of the different shapes and sizes was counted at the levels of the reticular formation examined.

The results of the photometric and microscopic investigations were compared (Figs. 1 and 2).

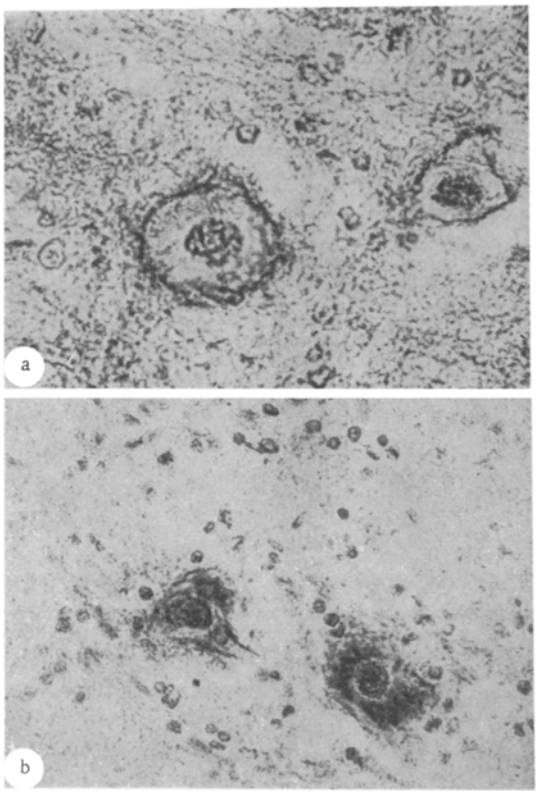

Fig. 3. Different types of reticular cells containing ACE: a) cells with strongest staining near border (400×); b) cells with high cholinesterase activity, cytoplasm strongly stained (200×).

EXPERIMENTAL RESULTS AND DISCUSSION

ACE was detected at all investigated levels of the medulla from the middle third of the facial nerve nucleus to the middle third of the inferior olive. Topographic schemes of distribution of ACE activity in the section, based on the results of photometry (Fig. 1), and schemes of localization of cells possessing cholinesterase activity, based on the results of microscopic examination of these sections (Fig. 2), were prepared for all levels. Specific nuclei of the medulla (nuclei of the cranial nerves, inferior olives, etc.) were characterized by bright staining, and the photometric values varied from 18 to 60 c.u. (the maximum). A low content of the enzyme (from 0 to 10 c.u.) was found in the region of the spinal tract of the trigeminal nerve and the dorsal spinocerebellar and spinothalamic tracts. The rubrospinal and vestibulospinal tracts gave a high intensity of staining, measuring 14-30 c.u.

The intensity of staining in the nuclei of the reticular formation was low: the few darkest areas corresponded to 25 c.u. The parvocellular, gigantocellular, and ventral reticular nuclei, vestibular nuclei, and zone of the tractus solitarius were investigated in greatest detail.

In the parvocellular reticular nucleus the largest amounts of ACE were found in the dorsal and ventral parts, where the intensity of staining reached 20 c.u. (against a general background of 12-14 c.u.), along the border with the nucleus of the spinal tract of the trigeminal nerve, and at the level of the middle third of the inferior olive, along the border with the nucleus of the tractus solitarius. Comparison of the results of investigations of sections stained with toluidine blue with the histochemical data showed that only a few of the large cells (40-50 μ or more) in the parvocellular nucleus contain ACE, and that most of the cells which stained were triangular in shape. Only a relatively small proportion of the small cells, which constitute the bulk of this nucleus, gave a positive reaction for ACE, and most of the cells which were stained were triangular or oval in shape.

During photometry of the ventral nucleus the ACE content was equivalent to an intensity of staining of, on the average, 12-16 c.u.. An increase in the intensity of staining (to 20-25 c.u.) was observed in the ventral and ventrolateral regions of the nucleus, including the region of the nucleus ambiguus, and also along the medial and lateral borders of the nucleus. Microscopic examination showed that the cholinesterase activity is located mainly in small, oval-shaped cells. Large neurons gave a positive reaction for ACE relatively less frequently.

In the gigantocellular reticular nucleus ACE was localized mainly in the ventral part (along the border with the medial lemniscus, with the ventribulospinal tract, and with the nucleus of the facial nerve), and to a lesser extent in the dorsal parts, giving an intensity of 18-25 c.u. compared with the general background of 14 c.u.. Intensively stained zones also were found at the border with the parvocellular nucleus and in the medial part of the gigantocellular nucleus (Fig. 1). In the gigantocellular nucleus (just as in the other nuclei), the number of small cells staining for ACE was small by comparison with their total number; these cells were mainly oval in shape (Fig. 2). Most of the large neurons which gave a positive reaction for ACE were oval or fusiform, while in the more rostral parts of the nucleus stellate neurons also showed up. The total number of cells possessing acetylcholinesterase activity increased in the rostral direction.

In all the reticular nuclei investigated, some increase in the ACE content was observed on the border with the structures surrounding these nuclei.

In the vestibular spinal nucleus the ACE content was higher throughout its extent than in the medial nucleus. In the caudal parts of the vestibular spinal nucleus fields with high cholinesterase activity were located mainly in the dorsal, dorsolateral, and medial parts of the nucleus. In the rostral parts of the nucleus, areas of high cholinesterase activity were more common on the lateral than on the medial side. In the vestibular medial nucleus intensively stained fields were few in number, and in sections at all investigated levels they were localized in the ventral part and along the lateral border of the nucleus. The remaining regions of the nucleus had a low content of active ACE.

In the rostral part of the nucleus of the tractus solitarius most fields were rated at 12-16 c.u., and only a few areas with a higher enzyme content were found. In the caudal parts (at the level of the upper and middle thirds of the inferior olive) the ACE content was sharply increased, and the results of measurements of all fields ranged from 20 to 24 c.u. or more.

Cells of the reticular formation staining postively for ACE differed from each other in the intensity of their staining. Some cells stained strongly, others weakly, and the latter were difficult to pick out against the background. The distribution of the enzyme inside the cell also varied: ACE could be found only along the cell border, or it could be detected in the cytoplasm of the neuron (Fig. 3a, b). The distribution of cells giving a positive reaction for ACE agreed completely with the topography of the most intensively stained parts of the nuclei, giving high photometric readings (see Figs. 1 and 2).

The differences in the intensity of staining of the individual medullary structures in the reaction for ACE revealed by these experiments correspond to observations made by other workers [5, 10]. This study of the distribution of ACE in different parts of each of the investigated nuclei demonstrates that the cholinesterase activity varies from one part to another. The localization of cells containing ACE in the parvocellular and gigantocellular nuclei coincides with the localization in these nuclei of neurons which are sensitive to application of acetylcholine [15].

LITERATURE CITED

1. S. V. Anichkov, Farmakol. i Toksikol., No. 3, 194 (1960).
2. A. A. Grantyn', in: Current Problems in the Pharmacology of the Reticular Formation and Synaptic Transmission [in Russian], Leningrad (1963), p. 165.
3. É. V. Zeimal', Farmakol. i Toksikol., No. 2, 157 (1963).
4. E. I. Il'ina-Kakueva, Byull. Éksperim. Biol. i Med., No. 10, 100 (1963).
5. R. Yu. Il'yuchenok and L. N. Nesterneko, Fiziol. Zh. SSSR, No. 10, 1177 (1965).
6. T. G. Raigorodskaya, in: Proceedings of the 28th Students' Scientific Conference of the 1st Leningrad Medical Institute [in Russian], Leningrad (1966), p. 98.
7. P. B. Bradley and Y. M. Wolstencroft, Ann. New York Acad. Sci., 142, No. 1, 15 (1967).
8. A. S. V. Burgen and L. M. Chipman, J. Physiol. (London), 114, 296 (1951).
9. M. A. Gerebtsoff, Acta Anat. (Basel), 19, 366 (1953).
10. R. L. Holmes and J. H. Wolstencroft, J. Physiol. (London), 175, No. 1, 55P (1964).
11. B. Metz, Am. J. Physiol., 102, 101 (1958).
12. B. Metz, Neurology (Minneapolis), 11, 37 (1961).
13. A. C. Palmer and A. P. Ellerkep, Quart. J. Exp. Physiol., 46, 515 (1961).
14. R. Pavlin, J. Neurochem., 12, 515 (1965).
15. C. G. Salmioraghi and F. A. Steiner, J. Neurophysiol., 26, 581 (1963).