# COMPARATIVE HISTOCHEMICAL STUDY OF THE NUCLEI OF THE SPECIFIC SYSTEMS AND RETICULAR FORMATION OF THE BRAIN IN A SERIES OF MAMMALS

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Recent experimental findings have demonstrated the functional and structural nonhomogeneity of the reticular formation of the brain. Olszewski and co-workers [10, 11] concluded from their studies of the cytoarchitectonics of the reticular formation in different animals that this formation is not a morphological entity, but a collection of nuclei of varied structure. The evolutional method of investigation revealed that the principal trend in the development of the reticular formation is an increase in the degree of its structural homogeneity and specialization [1, 2, 9]. Physiological investigations have drawn attention to the great variety of functions performed by the reticular formation. Their results show that the reticular formation of the brain stem is a part of the so-called ascending activating system, interconnected with the cerebellum, and also that it influences spinal cord function. Meanwhile, there is an almost total absence of facts relating to the chemical organization of the reticular formation. Information of this sort would be particular interesting to the comparative study of the chemism of the nuclei of the reticular formation and the nuclei of the specific systems whose functions have been adequately studied.

It therefore appeared useful to study some aspects of the metabolic processes in the reticular formation, by means of histochemical methods. It was decided to conduct investigations in animals possessing different levels of complexity of the organization of their nervous system, for in recent years discussions have been held on the role of the reticular formation in the course of phylogenetic evolution [3, 4, 7].

## EXPERIMENTAL METHOD

The nuclei of the reticular formation of the medulla in mammals of different orders – the hedgehog, rat, cat, and monkey (Macacus rhesus) – were investigated. The succinate dehydrogenase (SDH) activity was studied by Nachlas's method using the tetrazolium nitro-BT as hydrogen acceptor, and the acetylcholinesterase (ACE) activity was investigated by Gomori's method, using acetylcholine iodide as substrate and di-isopropylfluorophosphate as pseudocholinesterase inhibitor. The following reticular nuclei were examined: central, giant-cell, small-cell, paramedian, and nucleus of the lateral funiculus (lateral nucleus). The following nuclei of the specific systems were chosen: the lateral group of cells of the anterior horn of the spinal cord, the nucleus of the hypoglossal nerve, and the relay nuclei of Goll and Burdach.

## EXPERIMENTAL RESULTS

The investigation showed that the homonymous cell formations of animals belonging to different mammalian orders differ in their SDH and ACE activity. The highest SDH and ACE activity was found in the nerve tissue structures of the hedgehog. A slightly lower level was found in the rat, lower still in the cat, and the lowest activity of all was found in the monkey. The difference between the SDH activity of the hedgehog and monkey was very marked, while the difference in ACE activity was less marked, and mainly observed in the nuclei of the specific systems. Why this is so difficult to explain. According to E. M. Kreps and N. A. Verzhbinskaya [6], the energy metabolism in the vertebrate brain undergoes considerable changes in the course of evolution, as manifested notably by an improvement in oxidative metabolism. These workers consider that the higher coefficient of useful action of the enzyme

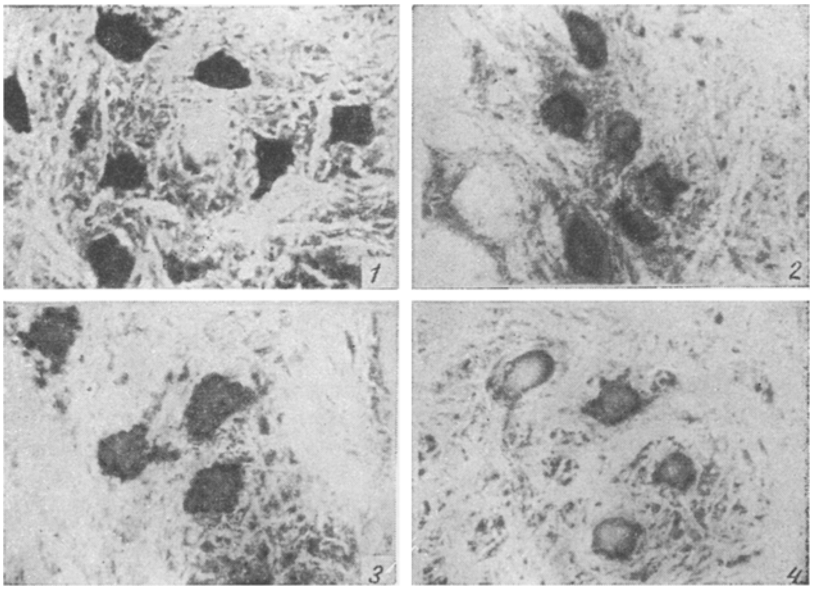

Fig. 1. Distribution of ACE activity in the structures of the anterior horns of the spinal cord. The fall in enzyme activity in the bodies of the motor neurons is clearly seen in the series: hedgehog (1) – rat (2) – cat (3) – monkey (4). Photomicrograph. × 20. Ocular Homal 6.

in the more highly organized animals enables the same effect to be obtained with a low concentration of substances as in animals with a lower level of organization with high concentrations of biologically active substances. This evidently explains the lowered activity of the enzymes observed in the series of mammals.

The investigation also showed that the SDH and ACE activity in the reticular nuclei and the nuclei subserving a specific function differed significantly. The activity of these enzymes was always higher in the specific nuclei. The distinguishing feature of the distribution of SDH and ACE activity in the motor nuclei was the relatively high enzymic activity in the bodies of the neurons, compared with the activity in the structures surrounding the nerve cells. The difference between the levels of enzymic activity of individual neurons was small. The ACE activity was highest on the surface of the bodies of the nerve cells and in the bodies of the neuroglial cells. This relationship between the enzymic activity of the bodies of the nerve cells and the surrounding structures in the motor nuclei was characteristic of all species of animals investigated (Fig. 1).

It should be noted that the structures situated between the bodies of the neurons contained neuroglial elements and blood vessels, but consisted mainly of dendritic processes, so that when we speak of intercellular structures we mean mainly these processes, more especially since the SDH activity in the neuroglial elements corresponded, according to our observations, to the level of its activity in the dendritic processes.

In the relay nuclei of Goll and Burdach many of the bodies of the neurons also possessed a higher level of SDH and ACE activity than the surrounding structures, but the level of enzymic activity was not the same in all the cells and, moreover, part of the bodies of the neurons possessed the same level of enzymic activity as the structures surrounding them.

Species differences in the distribution of activity were clearly observed only in respect of SDH. In the nuclei of Goll and Burdach in the hedgehog the SDH activity was high, and as a rule it was higher in the cell bodies than in the intervening structures. In the rat, in contrast to the other species of animals, the SDH activity in the cell bodies of these nuclei fell compared with the hedgehog to the level of activity of the intervening structures, so that the cells became difficult to distinguish in the sections; an exception to this was given by the bodies of isolated neurons, more often larger ones, which possessed higher activity. This distribution of SDH activity in the rat appears to be the expression of species differences. In the cat and monkey, animals at a higher level of evolutional development, a considerable fall in SDH activity was observed by comparison with the hedgehog and rat, mainly on account of the structures situated between the bodies of the neurons. The bodies of the cells in these nuclei possessed relatively high enzymic activity, as a result of which they stood out clearly against the palely stained intercellular structures. In the

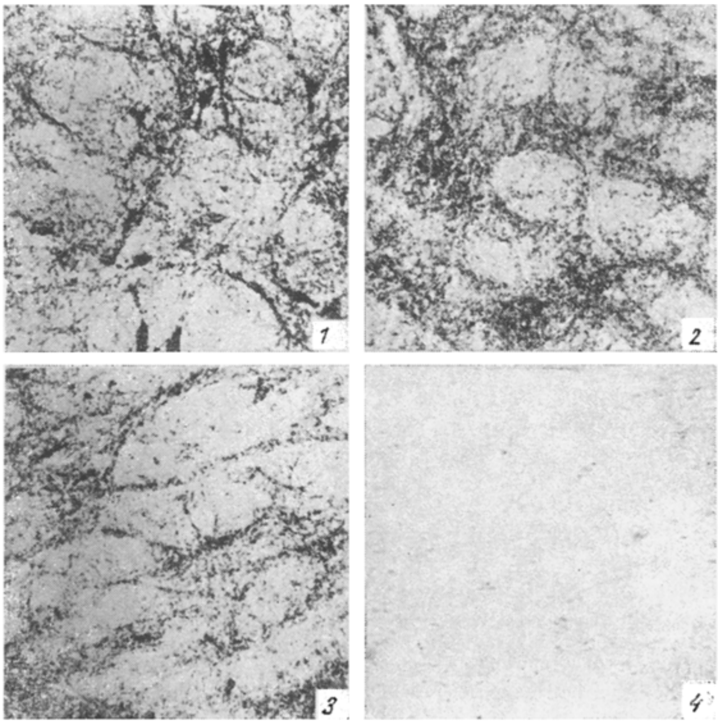

Fig. 2. Distribution of SDH activity in the structures of the small-cell reticular nucleus. It can be seen that in the series: hedgehog–rat-cat-monkey there is a significant lowering of enzyme activity, more marked in the bodies of the nerve cells than in their processes. Photomicrograph. x 20. Ocular Homal 6.

monkey, by comparison with the cat, the bodies of the nerve cells revealed great variation in activity: side by side with cells possessing relatively high SDH activity were others with average and low activity. The difference in the level of activity of the individual neurons was more marked in the nucleus of Goll than in that of Burdach. This fact may be related to functional differences arising in the course of evolution of the primates.

In accordance with the character of distribution of their SDH and ACE activity, the nuclei of the reticular nuclei that we investigated may be divided into two groups. The first group includes the central, giant-cell and small-cell nuclei. These nuclei may be called typical reticular nuclei. The second group includes the nuclei of the raphe, and the lateral and paramedian nuclei, and in their histochemical characteristics they are closely similar to the nuclei subserving a specific function. As regards their neuronal structure, these nuclei may also be classed as specific [5].

The comparative study of the SDH and ACE activity in the typical nuclei of the reticular formation shows that as the complexity of the nervous system increases in the series from the hedgehog to the monkey, changes take place in the character of distribution of SDH activity while the distribution of ACE remains unchanged.

Turning for a moment to the distribution of SDH activity in the typical reticular nuclei, it must be noted that in the hedgehog the activity in nearly all the bodies of the nerve cells in these nuclei was higher than the activity of the intervening structures. In the rat the SDH activity in most cell bodies fell to the level of activity of the intercellular structures; in some cells, however, mainly the large cells situated in the medial part of the central and giant-cell nuclei, and in all the giant cells, the activity of this enzyme remained higher. In the cat and monkey the distribution of SDH in these nuclei was similar to the distribution of this enzyme in the rat, but in the medial part of the central and giant-cell nuclei there were more neurons whose bodies possessed a higher SDH activity than in these nuclei in the rat. These were usually the larger nerve cells, although smaller neurons were to be found among them. It should be noted that in the cat and monkey these neurons showed greater variation in the level of enzymic activity. In the central and giant-cell nuclei groups of 3 or 4 cells were often seen, possessing equal enzymic activity.

Hence, comparison of the distribution of SDH in animals belonging to different orders shows that a redistribution of enzymic activity between the bodies of the neurons and their processes takes place in the reticular nuclei with increasing complexity of the nervous system (Fig. 2). At the same time differences between the medial and lateral regions of the reticular formation appeared and became more marked, as shown by the fact that the SDH activity of the medial part remained higher than that of the lateral part, against the background of a general lowering of enzymic activity in the series from hedgehog to monkey. This phenomenon may possibly be associated with the differentiation of the reticular formation in the process of evolution into regions with different functions.

If Friede [8] is right when he suggests that greater enzyme activity in the bodies of the neurons than in the neuropil is evidence of an efferent function, and that the opposite state of affairs is evidence of a receptive or associative function, then we may suppose that the cells with higher enzymic activity situated in the medial region of the reticular formation subserve an efferent function, while the cells of the lateral region, which possess the same level of SDH activity as the intercellular structures, fulfill a receptive or associative function.

In contrast to SDH, the distribution of ACE activity in the reticular nuclei of the species of animals investigated was basically the same. The typical reticular nuclei had a lower level of ACE activity than the specific nuclei. Characteristic findings were a low, or total absence of, ACE activity in the majority of neurons, including the giant neurons (except in the hedgehog, in which the giant cells showed relatively high ACE activity). Meanwhile, the reticular nuclei contained a few neurons whose bodies possessed higher enzymic activity than the surrounding structures. These cells were more numerous in the hedgehog and significantly fewer in the other species of animals. Cells of this type were situated in the reticular formation without any obvious regular pattern. In addition, in various parts of the reticular nuclei small areas of nervous tissue were found whose structures contained higher ACE activity than the remainder of the nucleus. The absence of ACE activity or its low level in the neurons of the nuclei of the reticular formation suggests that most of them are adrenergic structures, but with structures of a cholinergic nature among them.

## SUMMARY

Histochemical methods were used to study the comparative distribution of succinate dehydrogenase (SDH) and acetylcholinesterase (ACE) activity in the reticular formation nuclei of the predulla and in the "specific" formation of the medulla and the nuclei of Goll and Burdach in the spinal cord, the nucleus of the hypoglonal nerve and the motor nuclei of the spinal cord in mammals with a different complexity of organization of the nervous system . The activity of SDH and ACE differed in the representatives of various orders of mammals. It decreased in the series: hedgehog, rat, cat, monkey. The nuclei of the "specific" brain formations chiefly possessed higher enzymatic activity than the reticular nuclei and the activity distribution in them was the same in all the animals. In the reticular nuclei changes occurred in the correlation of the activity level between the neuron bodies and "intercellular" structures compared with the hedgehog, in other animals the activity in the nerve cell bodies fell to the level of enzyme activity in the structures surrounding the cells. A gradual division of reticular formations into lateral and medial areas becomes evident; the SDH activity was higher in the medial area structures. This process is evidently connected with the functional differentiation of the reticular formation of the medulla during evolution.

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**All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.**