
RELATIVE NUMBERS OF NEURONS WITH DIFFERENT MEDIATOR MECHANISMS
IN MOTOR NUCLEI OF THE CAT CERVICAL SPINAL CORD

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Motor nuclei of the cat cervical spinal cord are formed by groups of neurons differing in their mediator metabolism. From 40 to 65% are true motor (cholinergic) neurons. The localization of the precipitate of the reaction for acetylcholinesterase in the perinuclear space, on the membranes of the granular reticulum, axolemma, neurofilaments, and neurotubules of the axons, and in the synaptosomes and synaptic space are evidence of the possible perinuclear synthesis of the enzyme and of its transport with the flow of axoplasm. Comparison with the autoradiographic detection of glycine showed that large motor neurons form groups with small short-axon glycine-containing neurons, which make contact with them. The motor neurons have polyreceptive properties, for endings containing cholinesterase, glycine, noradrenalin, and serotonin, as well as unidentified endings are present on their soma and processes.

INTRODUCTION

The cholinergic nature of structures of the nervous system is nowadays identified histochemically by the detection of acetylcholinesterase (AChE). This indirect method is virtually the only one, for no histochemical method is yet available for the detection of acetylcholine and the histochemical determination of cholinacetylase is extremely difficult and inadequately specific (for simultaneously with the enzymes, the accompanying coenzyme is also revealed by the reaction and, in addition, the water-soluble precipitate can diffuse from the site of the enzyme). The validity of the method of determination of acetylcholinesterase for the identification of cholinergic structures is confirmed by the results of
biochemical investigations which have shown that cholinacetylase and acetylcholinesterase are always present in all parts of the brain which contain acetylcholine. Changes in the concentration of the mediator and its enzymes run parallel to each other and in the same direction. Histochemical evidence of the cholinergic nature of neurons is given by the presence of a strong reaction for acetylcholinesterase in their cytoplasm and at sites on the cytoplasmic membrane and axolemma.

The cholinergic nature of the spinal motoneurons has been established by both light and electron microscopy by means of various modifications of the method of AChE determination [5, 8-11, 13]. It was observed during these determinations that the reaction product is absent in some neurons of the motor nuclei and, for that reason, they cannot be classed as cholinergic cells [5, 13]. An explanation of this fact was provided by the discovery of neurons containing glycine [6, 12] and gamma-aminobutyric acid [12] in the gray matter of the spinal cord. The object of the present investigation was to demonstrate the mediator nature of neurons forming functional groups in the motor nuclei of the cat spinal cord.

EXPERIMENTAL METHOD

AChE was detected by the Karnovsky--Roots method (1964) in longitudinal and transverse serial sections through the cervical segments C_2-C_8 of 14 adult intact cats. Neostigmine (10^{-6} \text{M}) and iso-OMPA (10^{-4} \text{M}) were added to the incubation medium as butyrylcholinesterase inhibitors. Butyrylcholinesterase was determined in parallel sections as the control. During the reaction for AChE a specific precipitate of copper sulfide was formed. It varied from yellow to dark brown and the density of distribution of the precipitate in the cytoplasm reflected the intensity of the reaction of AChE and the quantity of the enzyme in the structure concerned. Some of the material was embedded in epoxide resin and unstained ultrathin sections were examined in the electron microscope.

Noradrenergic and serotoninergic neurons were detected in experiments on five animals by a luminescence histochemical method [2]. To differentiate any possible autoluminescence, some sections were treated with formaldehyde vapor without incubation. A parallel control was set up for the localization of monoamine oxidase. Since this enzyme must be present in structures containing monoamines, its discovery is further confirmation of the presence of monoamines.

To detect glycine-containing structures in the cervical spinal cord of the cat an autoradiographic method was used. Frozen transverse sections (25 \mu m) through segments C_6-C_7, were incubated in cold Hanks' solution for 10 min, and then transferred to a solution containing [3H]glycine (4\cdot10^{-4} \text{M}, \text{specific activity} 100 \mu \text{Ci/g}). After incubation in this medium for 10, 20, and 30 min followed by rinsing in fresh Hanks' solution the sections were fixed in 4\% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and then mounted on slides. After coating with M emulsion the specimens were exposed in darkness at 4°C for 14 days. The sections were developed, mounted in balsam, and examined under the optical microscope.

EXPERIMENTAL RESULTS AND DISCUSSION

Twenty transverse serial sections each from the rostral and caudal portions of segments C_5-C_8 and 20 longitudinal serial sections each at different depths of the anterior horns were examined under the light microscope.

In most neurons of the anterior horn of the cervical enlargement the reaction for AChE was positive. The reaction was found in the perikaryon (from pale yellow to dark yellow in color) and it was weaker in the processes. A strong reaction for AChE was observed in the terminals. In this region there were many "boutons terminaux" containing AChE and also pre-terminal "boutons de passage." They were found on the bodies and processes of the neurons and in the neuropil. These terminals made contact with neurons containing and not containing AChE. According to data in the literature, this distribution of the reaction product in the perikaryon and processes of the neurons is evidence of their cholinergic nature [9].

The motor nuclei always contain cells with no reaction for AChE. These cells were classed as noncholinergic neurons.

The results of the electron-microscopic study confirmed the above findings. In electron micrographs obtained after the specific reaction for AChE neurons containing electron-dense

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