HISTOCHEMICAL IDENTIFICATION OF NEURONS INVOLVED IN CHOLINERGIC SYNAPTIC TRANSMISSION

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Histochemical methods have not been sufficiently used to identify neurons involved in cholinergic synaptic transmission. Further progress in this field depends on methods capable of determining the cholinergic and choliniceptive functions of a neuron in the same histological section.

In the writers' view, an approach to the solution of this problem is given by histochemical investigations of choline-acetyltransferase (CAT), which synthesizes acetylcholine (ACh) in neurons.

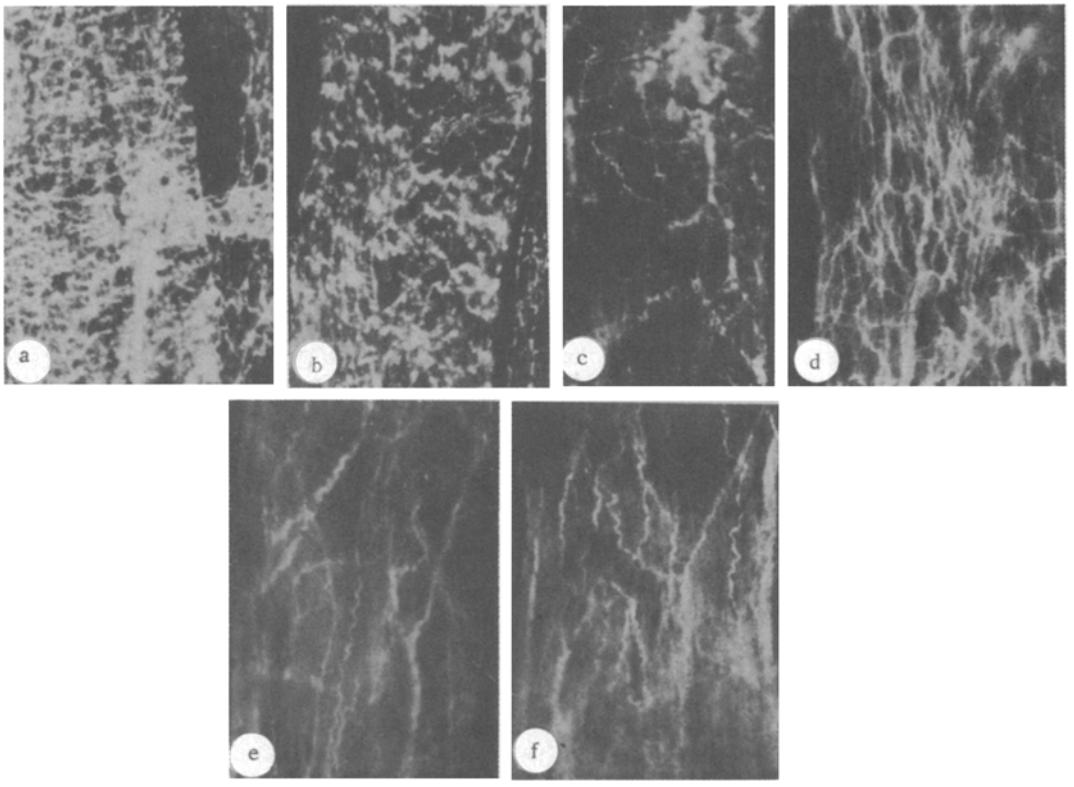

Fig. 1. Neurons of the human medulla: a) cholinergic choliniceptive neuron, synaptic boutons clearly visible on the right; b) cholinergic noncholiniceptive neuron; c) noncholinergic choliniceptive neuron, cholinergic axons converge on neurons (light ground); d) neurons of nucleus gracilius, high CAT activity in synaptic terminals. Stained by Burt's method, photomicrography, 1200 ×.

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EXPERIMENTAL METHOD

Neurons in medullary nuclei of five human fetuses at 22-27 weeks of intrauterine development were studied. Biochemical tests showed that CAT activity is considerable in all parts of the human brain at this age.

The principal reagents: acetyl-CoA, choline chloride, sodium cacodylate, and diisopropylfluorophosphate (DFP) were obtained from Calbiochem, USA. The lead nitrate and sucrose were recrystallized before use. Buffer solutions were made up in bidistilled and deionized water. Transverse slabs of medulla 4-5 mm thick were fixed in 1% formaldehyde solution, made up in 0.1 M cacodylate buffer with 0.32 M sucrose, at 4°C for 2 h, and rinsed in cacodylate buffer, pH 5.2. Sections 10 μ thick, cut in a cryostat, were mounted on coverslips, dried in air, and put into propylene cuvettes with ground stoppers. Nonspecific processes were inhibited by DFP [4]. Into each cuvette containing the specimens 0.5 ml of preincubation medium (pH 6), cooled to 4°C, and containing 2 mM DFP, 10% sucrose, and 25 mM cacodylate buffer, was added to them. The final concentration and composition of the medium were as follows: 25 mM cacodylate buffer, pH 6.0, 1 mM DFP, 4 mM choline chloride, 1-2 mM lead nitrate, 5% sucrose, 0.3 mM acetyl-CoA. The incubation medium with brain sections was incubated for 2.5 h at 37°C. The sections were then washed with water, treated with 5% ammonium sulfide, and mounted in balsam.

EXPERIMENTAL RESULTS

The localization of CAT in the neuron was revealed by the precipitate formed as a result of the reaction, and its color and extent reflected activity of the enzyme [1]. This characteristic of neurons in human medullary nuclei was described by the writers previously [2]. In this communication criteria are given whereby the possible identity of neurons concerned in cholinergic synaptic transmission can be identified. Two features characterizing either the choliniceptive or cholinergic function of a neuron can be distinguished. These features are as follows: a) the presence of CAT in the cytoplasm and processes is evidence of ACh synthesis in that neuron and confirms its cholinergic function; b) the presence of CAT in synaptic structures proves, on the one hand, that ACh is synthesized in them and, on the other hand, that the neuron with which these synapses make contact is choliniceptive.

Combined and separate analysis of these two features enabled three types of neurons concerned in cholinergic transmission to be identified. The first type, the cholinergic-choliniceptive neuron (Fig. 1a), is characterized by high CAT activity in the cytoplasm and by the presence of cholinergic synapses on its body, which correspond to acetylcholine receptors of the plasma membrane. This type of neuron was discovered in all nuclei of the cranial nerves. The second type, the cholinergic noncholiniceptive neuron (Fig. 1b) has high cytoplasmic CAT activity. It has no cholinergic synapses on its body or processes and, as data in the literature show, it may be serotonin- [5], dopamine- [7], noradrenalin- [8], or GABA-receptive [6]. Cholinergic noncholiniceptive neurons were found in all nuclei investigated in the human medulla [2]. The third type, the noncholinergic choliniceptive neuron (Fig. 1c) has no CAT and does not synthesize ACh; it receives cholinergic impulses from CAT-rich synapses; it carries cholinergic terminals on its body and processes. Synaptic terminals were found particularly effectively in the neuropil of sensory nuclei (Fig. 1d).

The method for CAT can be used to study conducting fibers in the brain with a cholinergic function and connections between neurons synthesizing acetylcholine for transmission of the nervous impulse. Comparison of results obtained by histochemical investigation of CAT [3], on the one hand, and by a quantitative radiochemical method [7], on the other hand, ensures high reliability of the results.

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