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LOCALIZATION OF CHOLINE ACETYLTRANSFERASE IN HUMAN BRAIN STEM NEURONS

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KEY WORDS: choline acetyltransferase; brain stem; cholinergic-choliniceptive, cholinergic-noncholiniceptive, and noncholinergic-choliniceptive neurons.

There is as yet no unanimity among research workers on the relative importance in the human brain of nervous connections along which impulses are transmitted during acetylcholine (ACh) secretion [1]. Identification of the mediator in brain interneurons proved particularly difficult. This difficulty was not overcome by the use of a radiometric method, such as is widely used to study cholinergic transmission [5]. Real opportunities for establishing the topography of Ach-synthesizing neurons have been provided by histochemical and immunohistochemical methods of investigation of choline acetyltransferase – CAT (E.C. 2.3.1.6). These methods have the important advantage that cholinergic and choliniceptive functions of the neuron can be diagnosed simultaneously in the same histologic preparation [7].

EXPERIMENTAL METHOD

CAT activity was studied in the medulla, pons, and midbrain of five human fetuses aged 6-8 lunar months. By this time, according to data in the literature [6], the enzyme has attained definitive activity. A full description of the histochemical method of CAT detection was given previously [3]. Transverse sections 10 μ thick of the brain stem were cut in a cryostat, and incubated at 37°C for 2.5 h in solution with the following final concentration: 25 mM cacodylate buffer, $1 \cdot 10^{-3}$ M DFP, 4 mM choline chloride, 1 mM lead nitrate, 5% sucrose, and 0.3 mM acetyl-CoA, pH 6.0. After incubation the sections were washed with water, treated with 5% ammonium sulfide, and mounted in balsam. The sections were studied under maximal resolving power of the light microscope, using an immersion system so that cytoplasmic and synaptic CAT could be determined.

EXPERIMENTAL RESULTS

The localization of CAT was determined by the precipitate formed as the result of the reaction, the color and extent of which indicated the level of enzyme activity [2]. The enzyme was discovered in the cytoplasm and processes of neurons and also in synaptic terminals (Fig. 1). On the basis of these signs, the choliniceptive and cholinergic functions of the neuron could be identified. The presence of CAT in the cytoplasm and processes is evidence of ACh synthesis in the given neuron and indicates that it has a cholinergic function. The localization of CAT in the synaptic thickenings 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 has choliniceptive function.

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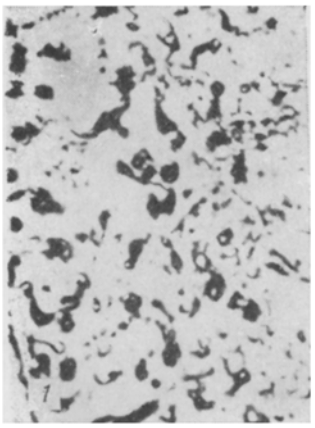

Fig. 1

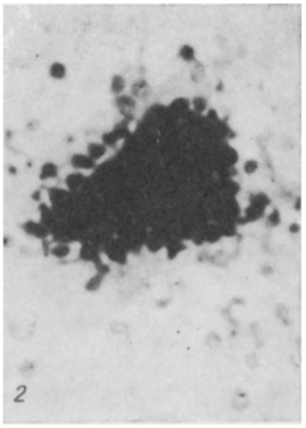

Fig. 2

Fig. 1. Neuropil of sensory nucleus of trigeminal nerve: high CAT activity in synaptic terminals. Here and in Figs. 2-4, Burt's method; 1000 ×.

Fig. 2. Cholinergic-choliniceptive neuron of superior olivary complex.

Fig. 3

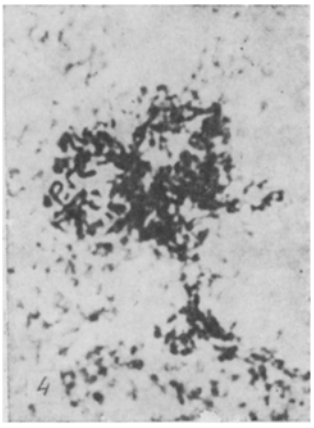

Fig. 4

Fig. 3. Cholinergic-noncholiniceptive neurons of superior salivary nucleus.

Fig. 4. Noncholinergic-choliniceptive neurons of locus coeruleus.

Combined and separate analysis of these features led to the differentiation of three types of neurons concerned in cholinergic transmission (Table 1). The first type is the cholinergic-choliniceptive neuron (Fig. 2). It is characterized by a high level of CAT activity in the cytoplasm and processes, and by the presence of cholinergic synapses on its body to which correspond the acetylcholine receptors of the plasma membrane. Neurons of this type have been identified in the nucleus ambiguus, nuclei of the abducens, facial, trochlear, and oculomotor nerves, in the superior olivary complex, nucleus of the trapezoid body, and in some large neurons of the reticular formation. The second type is the cholinergic-noncholiniceptive neuron (Fig. 3). This possesses high cytoplasmic CAT activity but has no cholinergic synapses on its body or processes. Among centers containing a large number (++) of cholinergic-noncholiniceptive neurons are the nucleus of the hypoglossal nerve, supraspinal nucleus, dorsal nucleus of the vagus nerve, nucleus ambiguus, lateral vestibular nucleus, nucleus of the facial nerve, superior salivary nucleus, nucleus of the abducens nerve, motor nucleus of the trigeminal nerve, nucleus of the trochlear nerve, Perlia's nucleus, Westphal—Edinger nucleus, principal nucleus of the oculomotor nerve, superior olivary complex, nucleus of the trapezoid body, nucleus of the lateral lemniscus, and large cells of the reticular nuclei: pedunculopontine nucleus, parabrachial pigmented nucleus, and gigantocellular nuclei of medulla and pons. The third type is the noncholinergic-choliniceptive neurons (Fig. 4). This is

TABLE 1. Distribution of Neurons of Cholinergic Synaptic Transmission in Human Brain Stem Nuclei

Test object	Cholinergic-cholinoceptive neuron	Cholinergic-noncholinoceptive neuron	Noncholinergic-cholinoceptive neuron
Nuclei			
of Perlia	+	++	+
of Westphal-Edinger	+	++	+
principal of nerve III	+	++	+
of cochlear nerve	+	++	+
motor, of V nerve	—	++	—
sensory, of V nerve	—	—	++
of abducens nerve	+	++	+
of facial nerve	+	++	+
superior salivary	—	++	—
medial vestibular	—	—	++
lateral vestibular	—	++	+
dorsal cochlear	—	—	++
dorsal, of X nerve	—	++	+
of Tractus solitarius	—	—	++
ambiguus	+	++	—
supraspinal	—	++	—
of hypoglossal nerve	—	++	—
arcuate	—	—	++
of inferior olive	—	—	++
of superior olive	+	++	+
Nuclei pontis	—	—	++
Nucleus:			
of superior colliculus	—	—	++
of inferior colliculus	—	—	++
gigantocellular reticular	—	++	+
lateral reticular	+	++	+
parvocellular reticular	—	—	++
Nuclei raphe	—	—	++

characterized by absence of CAT in the cytoplasm and by the presence of many cholinergic terminals on its body and processes. Many noncholinergic-choliniceptive cells are found in the nucleus of the tractus solitarius, the medial, inferior, and superior vestibular nuclei, dorsal cochlear nucleus, sensory nuclei of the trigeminal nerve, nucleus gracilis, medial cuneate nucleus, inferior olivary complex, nuclei pontis, nuclei of the superior and inferior colliculi, interpeduncular and arcuate nuclei, nuclei raphe, red nucleus, nucleus coeruleus and nucleus subcoeruleus, nucleus compactus of the substantia nigra, and parvocellular nucleus of the reticular formation.

The nuclei studied contain mainly (++) either cholinergic-noncholiniceptive or noncholinergic-choliniceptive neurons. Far fewer nuclei contain all three types of nerve cells. It is a particularly interesting fact that noncholinergic-choliniceptive neurons are found in small numbers (+) in the motor nuclei of the cranial nerves. Incidentally, it is not always possible to distinguish a choliniceptive neuron on whose body a very large number of terminals may sometimes be found from a cholinergic neuron. Identification of neurons of cholinergic synaptic transmission in the mammalian brain therefore requires further study with the use of other methods of investigation [4].

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