Histochemical and cytochemical observations on the acetylcholinesterase (AChE) localization in the superior cervical ganglion (SCG) after preganglionic sympathectomy of the neonatal rat

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Summary. Results of histochemical and cytochemical reactions show that after the section of preganglionic nerve trunk performed in newborn animals, the appearance and the individual increase in AChE activity in non-innervated nerve cells follow the pattern of normally innervated ganglion cell.

In the innervated rat SCG, AChE (acetylcholine hydrolase, EC 3.1.1.7) is distributed between presynaptic axons and ganglion cells. The individual contribution of these 2 structures to the total AChE activity ist, however, not known. Considering the possibility that the preganglionic nerve exerts a trophic influence on the intracellular AChE[1-3], neither the quantitative assays of AChE activity in homogenized denervated ganglia[4,5], nor single cell activity determination[6] can provide reliable evidence for the real amount of intracellular enzyme in an innervated nerve cell. In the present study, an attempt was made to amplify the possible regulatory effect of presynaptic innervation on the ganglion cell AChE activity. For this purpose, the immature nerve cells were deprived of preganglionic contacts in the newborn rat when synaptic contacts are just beginning to form and AChE activity is still absent on growing presynaptic axons and negligible in immature ganglion cells[7,8].

Materials and methods. Albino rats (Wistar) of both sexes were used. The unilateral preganglionic denervation of the SCG was performed with sodium chloralhydrate (35 mg/100 g b.wt i.p.) anaesthesia in rats 1 or 2 days old. The ganglia were decentralized by the removal of 2-3 mm of preganglionic nerve trunk. The left untreated ganglion was used as a control. The animals were sacrificed with sodium chloralhydrate anaesthesia either 3 h, 1, 2, 7, days or 2, 3 or 4 weeks after operation. To avoid some significant changes due to mechanical or inflammatory disturbances, 'sham' operated animals were used as controls.

The halved, fixed ganglia (5% formaldehyde, pH = 7.4, overnight) were embedded in agar and sectioned into 30-µm-thick sections using Oxford vibratom sectioning system, model G. The first step of Cu-thiocholine technique[9] was employed for light microscopic localization of AChE. The tissue samples were incubated at pH 6.0 for 20 min with 2 mM acetylthiocholine iodide (Sigma, St. Louis) and 20 µM tetraisopropylpyrophosphoramide (iso-OMPA; Koch-Light, Colnbrook, Buck, England). Prior to incubation, the tissue was preincubated for 30 min in a histochemical medium containing the appropriate inhibitor without substrate.

For the electron microscopic localization of AChE, potassium thiocyanate modification[10] of Cu-thiocholine method[9] was used, except for pH which was adjusted to 6.0 with 20 mM maleate buffer solution. The sections were incubated for 10–15 min in a reaction mixture containing 2 mM acetylthiocholine chloride (Sigma, St. Louis) and 20 µM iso-OMPA. Control experiments for light and electron microscopic localization were performed with 20 µM eserine sulphate (BGD Chemical LTD Pools, England).

Results and discussion. When the section of preganglionic nerve trunk was performed in 1- or 2-day-old rats, the initial appearance and the subsequent development of AChE activity in non-innervated nerve cell bodies were in agreement with those in control ganglia in each developmental stage studied. Figure 1, a and b, shows 2 samples of 23-day-old rats; figure 1, b, represents the AChE activity 21 days after nerve section. Electron microscopic cytochemistry revealed the localization of the reaction product indicating AChE activity inside the cisternae of the rough endoplasmic reticulum of control (figure 2, a) and operated ganglia (figure 2, b). After the section of preganglionic nerve trunk of immature SCG, an unusually large amount of random reaction product was occasionally found distributed in the cytoplasm of young ganglion cells (figure 3, a); particles of enzyme product were also noted between plasmalemma and adjacent satellite cells (figure 3, b).

Thus, the appearance of AChE activity and the subsequent increase in the amount of the reaction product in the non-innervated ganglion cell closely resembled those in control

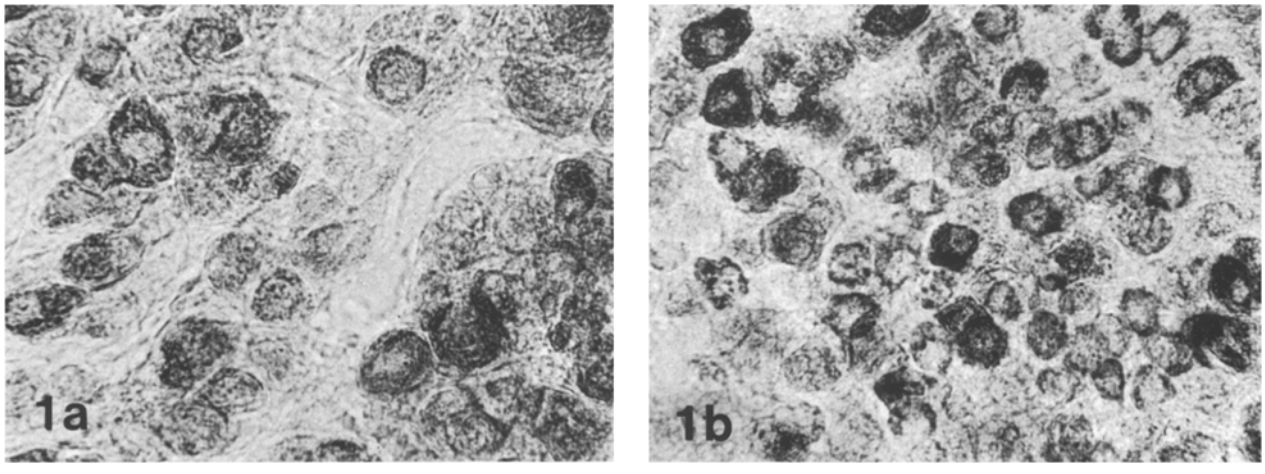

Fig. 1. Superior cervical ganglion. 23-day-old rat. Acetylthiocholine and iso-OMPA; incubation time 20 min. *a* The ganglion cell bodies of control ganglia exhibit different acetylcholinesterase activity. × 660. *b* 21 days after sympathectomy of 2-day-old rat. The distribution of the reaction product within the cytoplasm of non-innervated ganglion cells resembles that in control ganglia. × 510.

ganglia at the same developmental stage. It may be concluded that in nerve cells of which nerve contacts are not yet fully developed, the regulation of AChE synthesis is to a great extent independent on the afferent innervation. However, it had been established that a wide variety of experimental conditions led to a neuronally mediated increase of thyrosine hydroxylase and some other enzymes involved in the synthesis of catecholamines[11–15]. Thus, the possibility that the intact afferent input is essential for the subsequent maintenance of the intracellular AChE activity in adult rat SCG, could not be ruled out. But the possible effect of preganglionic innervation on the intracellular AChE activity might be studied only if the reliable distribution of enzyme activities between the extra- and intracellular structures in a normally innervated SCG of adult rat were known.

The random reaction product, seen often to be scattered throughout nerve cell cytoplasm, next to the localization on the intracellular membranes, may indicate that the ganglion cell deprived of nerve contacts has a decreased ability to retain its intracellular AChE[1]. Such a case has been postulated for brain and spinal cord neurons, where the firmness of membrane AChE binding predominantly depends on the intensity of afferent neuronal activity[16].

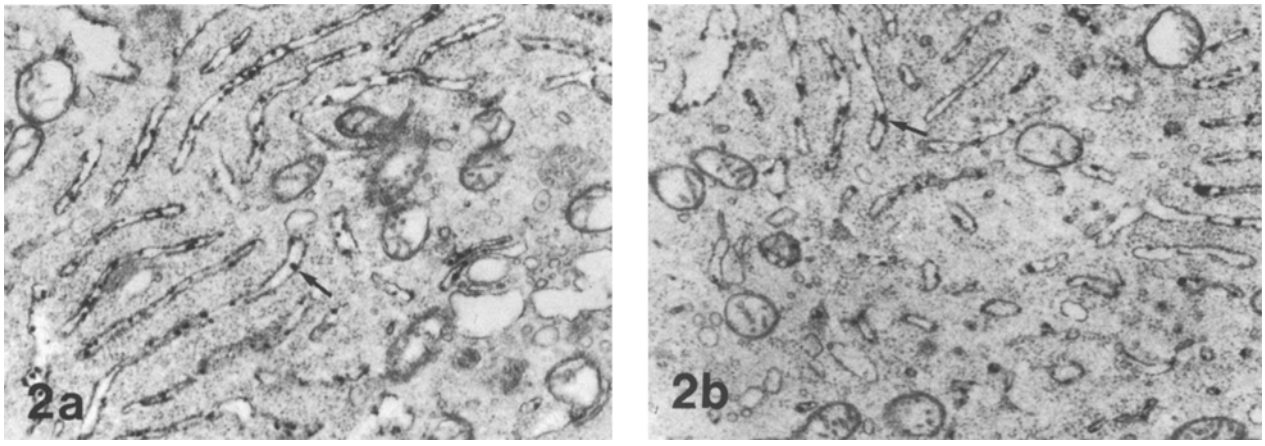

Fig. 2. Electron micrograph of the superior cervical ganglion. 23-day-old rat. Acetylthiocholine and iso-OMPA; incubation time 15 min. *a* Acetylcholinesterase activity is distributed in the cisternae of the endoplasmic reticulum (arrow) of ganglion cell. × 24,100. *b* 21 days after sympathectomy of a 2-day-old rat. The reaction product is bound in the cisternae of endoplasmic reticulum (arrow), similarly as in control ganglia. × 24,100.

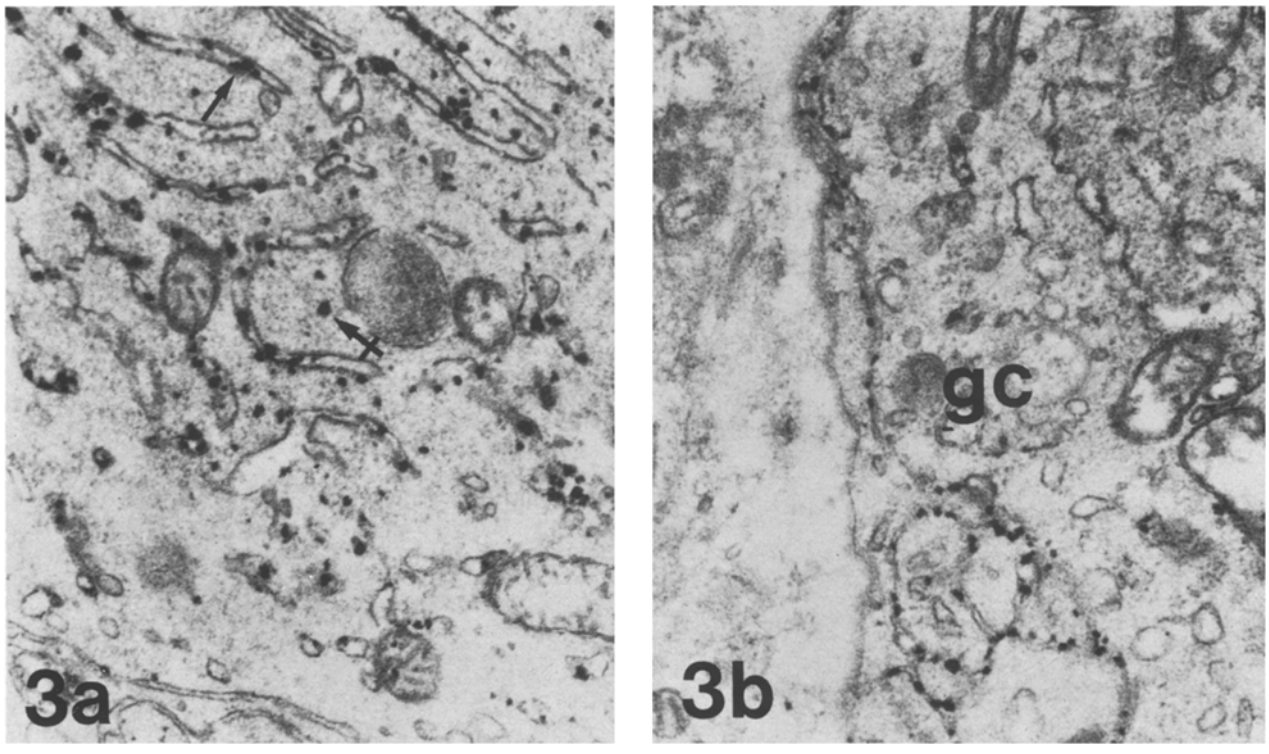

Fig. 3. Electron micrograph of the superior cervical ganglion. 23-day-old rat. 21 days after sympathectomy of a 2-day-old rat. Acetylthiocholine and iso-OMPA; incubation time 15 min. *a* Next to the localization on the intracellular membranes (arrow), the reaction product is seen to be scattered throughout nerve cell cytoplasm (crossed arrow). × 35,300. *b* Particles of reaction product are visible between ganglion cell (g c) plasmalemma and adjacent satellite cell. × 44,000.

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X-ray microanalysis in pearls of *Mytilus edulis* L. (Bivalvia)

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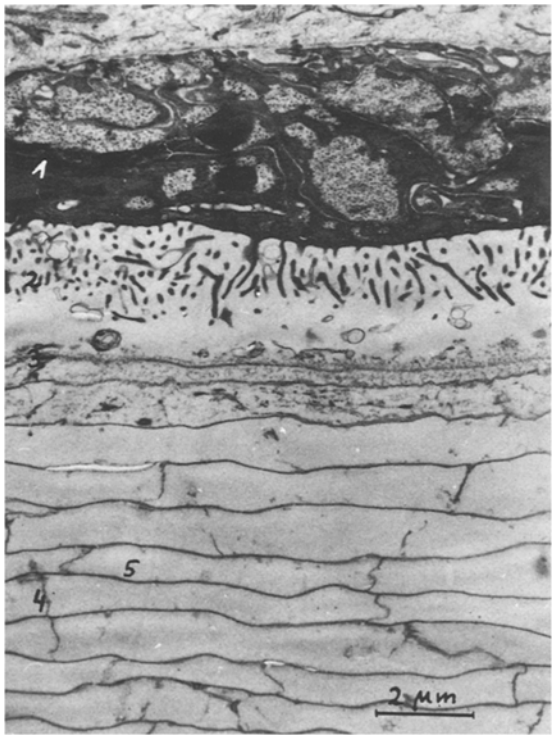
Summary. Metacercariae feeding on the tissues of Mytilus edulis deposit their excretes into the mussel. The clusters of excretes are surrounded by a 'pearl sac'. Increasing amounts of Ca-proteids are transported into the pearl sac epithelium and into the pearl.

In the common European bivalve Mytilus edulis L. the formation of pearls is induced by parasites. The metacercariae of a trematode (Gymnophallus choledochus) feed on the tissues of this mussel species and deposit clusters of excretes. The bivalve separates these excretes and surrounds them as well as dead metacercariae by conchin lamellae. The lamellae are arranged concentrically and radially, forming small pockets into which aragonite is deposited.



Electron micrograph of an ultrathin section through a small part of the pearl sac and the outer layers of a pearl (Mytilus edulis). *1* Epithelial cell of the pearl sac containing Ca-proteid complexes; *2* extracellular space with microvilli of the pearl sac epithelial cells; *3* outermost concentrical lamella of the pearl; *4* radial lamella; *5* pocket formed by concentrical and radial lamellae.

The accumulation of $CaCO_3$ in the tissues, the formation of a Ca-proteid complex and its transport into the pearl sac and into the pearl were studied by X-ray microanalysis in scanning electron microscopy, using a Siemens STEM 100 F[1].

The formation of a pearl starts as soon as the metacercaria has deposited its excrete cluster into the mussel tissue. Amoebocytes form the pearl sac which encloses the excrete products and which secretes mucopolysaccharides and proteids. The conchin lamellae originate from these organic substances by a condensation process. Several – at least 3 – concentric lamellae, as well as the radial lamellae are in simultaneous formation in the peripheral growing region of the pearl. After termination of the formation of the conchin lamellae, the crystallization of aragonite takes place. Probably, both substances, the proteids of the conchin and the $CaCO_3$ of the aragonite, originate from the same Ca-proteid complexes, which are traced in the cells surrounding the pearl. These complexes are found within smaller and larger vesicles in the pearl sac epithelium and in other adjacent tissues, too. They are not only different in their transmission electron microscopical appearance, but also in their chemical composition, as revealed by X-ray microanalysis. Especially, the contents of Ca, S, P, and Si are different. The occurrence of Ca-proteid complexes is not restricted to the area of pearl formation. According to the important role of the Ca-complexes in shell formation, shell repair and regulation of the pH, for example, they are also found within several other cell types of the molluscan body. But, immediately after a starting pearl formation, increasing amounts of Ca-proteids are transported to adjacent tissues of the pearl, into the pearl sac epithelium, and from there into the pearl. The calcium is transported as a complex form from the vesicles into the conchin pockets, passing the conchin lamellae which represent a network of very fine meshes (diameter about 25 nm[2,3]).

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