The acetylcholinesterase reaction and catecholamine fluorescence in the glomus cells of rat carotid body[1]

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Summary. The presence of both acetylcholinesterase reaction and glyoxylic acid-induced fluorescence of catecholamines in the same glomus cells of rat carotid body was demonstrated using combined histochemical methods. A suggestion is made that the glomus cells have both excitatory and inhibitory effects on the chemosensory nerve via acetylcholine and catecholamines, respectively.

The distribution of cholinesterases in the carotid body is well-documented[2,3]. The presence of catecholamines in carotid body glomus (type I) cells is also well-established[4]. The aim of the present study was to compare the distribution of the acetylcholinesterase (AChE) reaction and catecholamines in the carotid body, using 2 techniques for the combined demonstration of glyoxylic acid-induced fluorescence and cholinesterase reactions in the same specimen[5].

Carotid bodies of adult rats of the Sprague-Dawley strain were used in the present study. For the simultaneous demonstration of glyoxylic acid-induced fluorescence (GIF) and cholinesterase reactions, small pieces from the carotid body region were first immersed for 5 min in ice-cold 2% glyoxylic acid (GA) solution, pH 7.2. They were transferred to the preincubation solution and incubation solutions for cholinesterases made according to Karnovsky and Roots (1964). After incubation the specimens were rinsed in 2% GA solution for 30–60 min. They were then frozen in liquid nitrogen, freeze-dried and exposed to formaldehyde gas at 80 °C for 1 h and vacuum-embedded in paraffin.

For the demonstration of GIF and cholinesterase reactions consecutively in cryostat sections, the specimens were first fixed by combined perfusion and immersion with 2% GA solution. After immersion the specimens were frozen and sectioned with a cryostat. The cryostat sections were rinsed with the GA solution and dried with warm air from a hair dryer. The fluorescence was developed by prolonged warming or by formaldehyde gassing for 3 h at 37 °C, and it was then photographed. The cholinesterase reaction was then performed as above and the reaction product was photographed from the same site as before. Acetylthiocholine iodide was used as a substrate. 10^{-5} M iso-OMPA was used as a specific inhibitor for the nonspecific cholinesterase activity. 10^{-5} M Eserine was used to inhibit all the cholinesterase activity. The specimens were photographed using a Leitz Ortholux microscope equipped with devices for both fluorescence and transmitted light studies.

When pieces from carotid bodies were treated with GA solution and incubated as small pieces in the thiocholine media, they showed simultaneously a reaction product of acetylcholinesterase (AChE) reaction in the cell membrane of some fluorescent (type I) cells. In sections in which the GIF was first photographed and the AChE reaction then performed, the AChE reaction was located both at the cell membrane and there also seemed to be some activity in the cytoplasm of all fluorescent (type I) glomus cells (see figures).

Both methods clearly showed the presence of AChE-positive nerve fibres in the vicinity of the parenchymal cell groups and around blood vessels. The slightly different results obtained with these 2 methods may be due to differences in the penetration of the cholinesterase reagents. The consecutive reaction order when applied

1 Acknowledgments. The present study was supported by grants from the Emil Aaltonen Foundation and the Finnish Cancer Foundation to Timo Waris.

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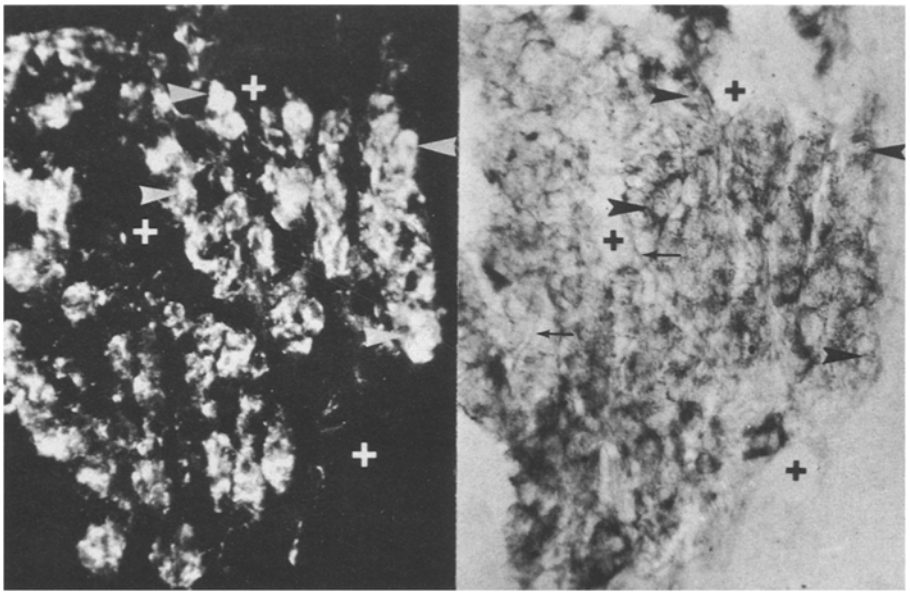

Fig. 1. A fluorescence photomicrograph, glyoxylic acid-induced fluorescence from a cryostat section of rat carotid body. ×210. Fig. 2. The same field as in figure 1 after consecutive demonstration of acetylcholinesterase activity (incubation time 2 h). Arrowheads in the figures indicate corresponding cell groups. Cross sections of some blood vessels are marked with +. The course of some AChE-positive nerve strands is marked with arrows. ×210.

to cryostat sections seemed more suitable for the demonstration of the AChE reaction in cells such as glomus cells, which also exhibit catecholamine fluorescence.

The presence of AChE reaction in the fluorescent glomus cells is in agreement with the concept that these cells belong to the APUD cells[6]. The present observations are also in agreement with the electron-microscopical findings of Papka[7] on the localization of the AChE in the glomus bodies of rabbit heart. In his study the AChE was localized in the glomus cell perinuclear cisternae, glomus cell endoplasmic reticulum, glomus cell membrane, at the axolemma of unmyelinated axons and around the synaptic terminals to the glomus cells. The presence of AChE in catecholamine-containing glomus cells can be explained in many ways[7]. However, this finding and the observation of Ballard and Jones[8] on the presence of choline acetyl transferase in glomus cells of cat carotid body and the finding of Fidone et al.[9] on the uptake of ^{3}H-choline by the glomus cells support the presence of active acetylcholine metabolism in the glomus cells. Acetylcholine might be synthetized and stored in the glomus cells and discharged from the cells, to have an excitatory action on the chemonsensory nerve ending. However, although acetylcholine does excite the chemonsensory nerve ending, the chemonsensory nerve ending itself most is probably not cholinergic because impulses produced with physiological stimuli are not abolished by the action of cholinergic blocking agents[10]. The carotid body contains large quantities of dopamine and noradrenaline[11]. The effect of catecholamines, especially that of dopamine, on the chemonsensory drive is inhibitive in nature[12].

The definite presence of both catecholamines and acetylcholinesterase activity in the same glomus (type I) cells of carotid body, as demonstrated in this study, leads us to suggest that the glomus cells might store both excitatory and inhibitory (modulating) transmitters which do influence the chemosensory nerve ending.

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Particle aggregates in plasma and intracellular membranes of toad bladder (granular cell)[1]

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Summary. Freeze-fracture of granular cells of toad urinary bladder (*Bufo marinus*) reveals the presence, hitherto undescribed, of intramembranous particle aggregates in intracytoplasmic structures (tubules, vacuoles and vesicles) both in resting and vasopressin-stimulated epithelia.

Amphibian bladders have long been used as privileged models for studying water permeability in tight epithelia and its modulation by neurohypophyseal hormones. Convergent evidence from transmission[4] and scanning electron microscopy[5] indicates that there is cellular specificity for the hydrosmotic effect of vasopressin, the apical membrane of the granular cells being the primary target of the hormone. Moreover, cell organelles such as lysosomal granules and their attendant enzymes[6], microtubules and microfilaments[7–10] and phenomena such as exocytosis[11], pinocytosis[12] and changes in membrane fluidity[13] appear to be associated with the increase in permeability to water.

Recently, characteristic organizational patterns of membrane particles in different cell types of toad bladder were revealed by the freeze-fracture technique[14–16]. In addition, conspicuous aggregates of particles were seen in frog[17,18] and toad bladders[19–22] challenged with oxytocin or vasopressin. So far, these morphological alterations have only been found in the P and E fracture faces[23] of the apical

1 This work was supported by grants No. 3.553.75, 3.120.77 and 3.1300.73 from the Swiss National Science Foundation.

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3 The authors are indebted to Mrs Marthe Sidler-Ansermet, Miss Jacqueline Rial and Mr Michel Bernard for valuable technical assistance.

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