THE LOCALIZATION OF SPECIFIC CHOLINESTERASE IN THE RETINA

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Dr. Hebb's observation that the optic nerve is devoid of significant concentrations of specific cholinesterase (ChE) and is therefore presumably noncholinergic is in agreement with our own findings (7). On the other hand there is considerable difference regarding the distribution of the enzyme in the retina as reported by her and associates (2), Francis (1) and ourselves, in spite of the fact that the three groups employed modifications of the same general method. Therefore, it seems appropriate to comment on the importance of certain details of the histochemical method, and to emphasize its limitations.

Originally, fresh frozen sections were incubated in a medium containing acetylthiocholine, copper glycinate and phosphate buffer saturated with copper thiocholine; after the deposition of copper thiocholine at the sites of ChE activity, the mercaptide was converted to copper sulfide (5). When pieces of detached rabbit retina were so treated and sectioned subsequently, the enzyme appeared to be concentrated in the nuclei of the bipolar cells (6), presenting a picture quite similar to that which Dr. Hebb has just described. In order to distinguish between specific (true, acetyl-, aceto-) and non-specific (pseudo, butyro-) ChE of cat tissues, DFP was used as a selective inhibitor and butyrylthiocholine as a selective substrate of the latter enzyme (3). Furthermore, it was found that diffusion of the enzyme occurred with the original procedure, resulting in a considerable degree of artifactual staining, particularly of nuclei, and that this factor could be controlled by incorporating the proper concentrations of sodium sulfate in the media (4). When fresh frozen sections of the cat's retina were reëxamined by this modified method, practically all the specific ChE was found to be confined to the amacrine cells (7). Francis (1), by incubating pieces of the retina of several species in media containing sodium sulfate and sectioning subsequently, found the same approximate distribution of the enzyme in the inner synaptic layer but interpreted it as being associated with the bipolar and ganglion cells.

In seeking to explain these discrepancies, it should be noted that the concentrations of DFP and sodium sulfate employed in the modified method were determined by manometric measurements of inhibition and recovery done with homogenates of cat tissues. In order to apply the procedure to other species with assurance of accuracy, similar determinations should be made first. Incubation of portions of tissue prior to sectioning raises the question of the rates and degrees of penetration of all reagents.

In known cholinergic neurons of the spinal cord and autonomic ganglia, specific ChE was found along the entire length of the dendrites, soma and axons (4). Although the possibility cannot be ruled out, it would be entirely inconsistent with this pattern if the enzyme should be present only in limited portions

of the bipolar cells, or that it should occur in the cell bodies of the ganglion cells but not in their axonal prolongations as the optic nerve. Furthermore, if the bipolar cells are considered the first afferent neurons and the rods and cones as special sensory cells analogous, for example, to chemoreceptor cells, the absence of specific ChE from the former parallels the situation in the great majority of the sensory neurons of the dorsal root, nodose and Gasserian ganglia.

The significance of the presence of specific ChE in the amacrine cells defies explanation at the present time just as does the cells' actual function. They are thought to convey impulses laterally between groups of ganglion cells and peripherally from ganglion cells to bipolar cells. Their effects upon subjective visual phenomena have been stated to constitute "the biggest remaining mystery in the physiology of the retina" (8).

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