HISTOCHEMICAL STUDIES ON ZINC OF THE HIPPOCAMPAL FORMATION IN THE MONKEY

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The hippocampal formation of monkey was studied by sulfide silver technique. Intense positive reaction to this method was found in the stratum radiatum of h_3 , h_4 and h_5 of the hippocampus, as well as the stratum multiforme of the fascia dentata. All these areas correspond to the endings of mossy fibers at the apical dendrite of pyramidal cells in the hippocampal formation.

The hippocampal formation consisting of the hippocampus and fascia dentata has recently received much attention morphologically and functionally as one of the components of the limbic system. It was Maske (7) who described interesting findings of specific histochemical reaction in these areas. He noted a fresh red reaction in the hippocampal formation in response to dithizon injection in vivo, explaining it by the presence of zinc in this area. Fleischhauer et al. (2) and Otsuka et al. (8,9) found similar response probably due to the presence of zinc in the hippocampal formation of various mammals by dithizon or sulfide silver method.

Any detailed studies, however, have scarcely been carried out on zinc distribution in the hippocampal formation of man or monkey. In the present study, therefore, results of examination of the hippocampal formation of the monkey by sulfide silver method are reported.

MATERIALS AND METHODS

Five monkeys were craniotomized immediately after induction of ravonal anesthesia to remove the hippocampal formation. The specimen was then fixed in alcohol saturated with hydrogen sulfide, embedded in paraffin, cut serially 8μ thick and stained by hematoxylin-eosin, toluidine blue and sulfide silver methods (13).

RESULTS

The hippocampal formation was divided cytoarchitecturally and myeloarchitecturally into several areas and layers (11,12,14). These areas and layers were also distinctly found in monkeys. In the hippocampal formation of monkeys, the stratum radiatum were stained only by sulfide silver method in h_1 and h_2 .

Pyramidal cells in h_3 and h_4 of the hippocampus mostly gave negative results in sulfide silver stain. Scattered dark-brown granules representing positive reaction were seen in the so-called neuropils between cells. Layers with intense positive reaction were seen in the stratum radiatum inside of pyramidal cell layer by sulfide silver method (Fig. 1). Along the surface of the apical dendrite of the pyramidal cells at this layers, dark-brown, granular bodies with positive reaction were demonstrated (Fig. 2). No other layers were stained. The h_5 of the hippocampus failed to show such a regular arrangement of pyramidal cells as in h_1 – h_4 . Cells were scattered around the hilus of fascia dentata, where the dark-brown substances with granular or fibrous appearance were dispersed. Granular cells in the fascia dentata were not stained by sulfide silver method at all. In the innermost layer of the fascia dentata facing the hilus or the stratum multiforme, an intense positive reaction was noted. At this area granular substance with intense positive reaction showed the islet-like accumulation. Other layers of the fascia dentata, especially the stratum moleculare showed medium staining intensity.

DISCUSSION

Maske (7), using guinea pig, dog, rabbit, rat and mouse and Fleischhauer et al. (2) using hedgehog, guinea pig, rabbit, rat, hamster, cat and dog demonstrated red stain of the specific layer of the hippocampal formation following intravenous injection of alkaline alcoholic solution of dithizon in each animal. Otsuka et al. (8,9) also reported intense positive reaction of certain area and layer of the hippocampal formation of mouse, rat, guinea pig, rabbit, cat, dog and goat by sulfide silver method. As to the monkey, however, the brief report of Friede (3) is the only one available.

In the hippocampal formation of monkeys under our observation, the site with intense positive reaction by sulfide silver method was almost the same as that in other animals. In layers with weak positive reaction in other animals, however, scarcely any stain was demonstrated in the monkey. This might indicate a difference in stainability to sulfide silver in various animals. The axons of the granular cells of the fascia dentata which were termed the mossy fibers by Ramon y Cajal (1) form characteristic synapses with the apical dendrite of the pyramidal cells of h_2 and h_3 . At the hilus of the fascia dentata, the portion with intense positive reaction to sulfide silver stain probably correspond to the endings of mossy fibers terminating at the dendrite of scattered pyramidal cells. By the use of sulfide silver method improved for electronmicroscopical use, deposition of zinc as silver granules was demonstrated within the endings of mossy fibers in the hippocampal formation of rat and rabbit (4,5,6). In the electronmicroscopic autoradiography with Zn^{65} , grains were also found within the endings of mossy fibers (10). Based on these studies, zinc demonstrated in the hippocampal formation is probably located within the nerve endings of mossy fibers, acting as neurotransmitter or related substances.

Since our present study indicated an approximate agreement in the mode of distribution of zinc in the hippocampal formation between monkey and other animals, zinc in the hippocampal formation is probably located within the endings of mossy fibers in monkeys, participating in neurotransmission.

FIG. 1. Hippocampal formation, frontal section. h2-h5: hippocampal fields, mf: mossy fiber of h₃, gr: stratum granulosum of fascia dentata, mol: stratum moleculare of fascia dentata, mul: stratum multiforme of fascia dentata, rad: stratum radiatum of h₂. $\times 20$

FIG. 2. Pyramidal cell of h₃. Along the surface of the apical dendrite (ad) of the pyramidal cells (pc), granules with positive reaction are demonstrated. $\times 240$

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