IMMUNOHISTOCHEMICAL DEMONSTRATION OF PROTEIN KINASE A IN THE RAT HIPPOCAMPUS

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Protein kinase A (PKA) was localized in the rat hippocampus by an immunohistochemical technique. The antibody was raised against the catalytic subunit of bovine heart PKA and cross-reacted with the brain enzyme, as could be revealed by immunoblotting. The strongest reaction was detected in the pyramidal and granular cell layers and in the lacunosomolecular layer of the hippocampus formation. The reaction in the radial layer was restricted to the dendrites of the pyramidal cells. In the other regions mainly glial cells were stained.

Many cellular processes are regulated by phosphorylation reactions. Therefore, the cells dispose of a number of different protein kinases, which are regulated by different mechanisms. The protein kinase A (PKA) (EC 2. 7. 1. 37) is regulated by the intracellular concentration of cyclic AMP and seems to be the only receptor molecule for cAMP (6,18). A lot of cellular responses, like gene expression (1, 2, 19), lipolysis (3), synaptic events (19) and so on are realized by the activation of the protein kinase A.

Whereas a great number of biochemical data about the enzyme is available in literature, only little histochemical results have been published. We tried to localize the PKA in the rat hippocampal formation immunocytochemically to investigate the distribution of the enzyme between the different layers of this brain area.

MATERIALS AND METHODS

The catalytic subunit of bovine heart PKA was used as antigen for an immunization, as described by Poeggel *et al.* (16). The cross reactivity of the rabbit antiserum with rat brain PKA was tested by immunoblotting.

Adult Wistar rats were anaesthetized and perfused with 4% freshly prepared paraformaldehyde in 50 mM phosphate buffered saline, pH 7.4. After removing the brain, the hippocampi were prepared and immersion fixed for 24 hr. Fifty μ m

Abbreviations: PKA: protein kinase A, PDE: cyclic nucleotide phosphodiesterase, ATP: adenosine triphosphate, cAMP: cyclic 3', 5'-adenosine monophosphate.

vibratome sections were cut and washed overnight. The slices were incubated for 1 hr in 1 : 50 diluted goat normal serum at room temperature and then transferred into the first antibody (dilution 1 : 1000–1 : 2000) for 24 hr at room temperature. This was followed by rinsing 3×15 min with buffer and incubation in 1 : 50 diluted goat-anti-rabbit anti-serum for 1 hr. The slices were washed again for 3×15 min and incubated with 1 : 500 diluted PAP-complex for 2 hr at room temperature. After a washing procedure of 3×15 min the peroxidase was developed with diaminobenzidine according to Sternberger (20). The specificity of the reaction was controlled by comparison with the rabbit normal serum and by preabsorption of the antiserum with the antigen. All dilutions and washing procedures were performed with 50 mM phosphate buffered saline pH 7.4.

RESULTS

A strong immunoreaction was found in the pyramidal cell layer of the hippocampus (Figs. 1, 2) and in the granular cell layer of the dentate gyrus (Fig. 3). The somata of the neurons and their dendrites were stained. Furthermore, the lacunosomolecular layer (Fig. 4) was reactive. In the stratum oriens many glial cells were stained, which mostly contacted capillaries (Fig. 5). In some neurons the nuclei were stained by the antiserum (Figs. 1, 3). In the CA3-field only the pyramidal cell somata and dendrites were stained, whereas the mossy fibers did not contain any reaction product.

Control reaction with a rabbit normal serum (Fig. 6) and with the antiserum, preabsorbed with antigen, were negative.

DISCUSSION

The knowledge of the occurrence and distribution of enzymes, controlling main physiological processes can help to increase our understanding of the regulation of cellular events. The protein kinase A is the only enzyme, functioning as a third messenger in the cAMP-cascade and increase or decreases the activity of its target proteins by phosphorylation processes (4, 12). The strongest immunohistochemical reaction of an antiserum against the catalytic subunit of PKA was found in the cellular layers of the hippocampi formation (Figs. 1–3) and in the region rich in synapses (Fig. 4). The somata of the neurons and their dendrites seem to contain the most substrate proteins of the PKA, like microtubules, histone H1, pyruvate kinase, K^+ -channels, proteins of the synaptic membranes, cholinergic receptors and much more (9, 10, 12, 19). The presence of PKA in the nucleus is often described (7, 8, 11) and several nuclear substrate proteins were found (5, 17). This is in agreement with the nuclear localization of PKA-like immunoreactivity (Figs. 2, 3). Besides the strong neuronal reaction, glial cells were often stained by the antiserum, especially astroglial cells, contacting capillaries (Fig. 5). The presence of other second messenger systems in glial cells as well as the presence of the cyclic nucleotide degrading enzyme was already histochemically described (13–15). Important glial cell-coupled processes seem to be under the control of the cyclic AMP system. In the CA3-field an immunohistochemical reaction was detected only in the somata and dendrites of the pyramidal cells, the mossy fibers were not stained. This points to a postsynaptic

FIG. 1. PKA-like immunoreaction in the CA1-field of the rat hippocampus. Note the strong reaction in the pyramidal cells (p), and their dendrites (d). $\times 180$

FIG. 2. Immunoreaction in the pyramidal cells of the CA3-field. Note the reaction in somata and dendrites. $\times 440$

FIG. 3. Staining of the granular cells of the dentate gyrus. Note the different reaction of the nuclei. $\times 180$

FIG. 4. Reaction of the antibody in the radial layer (r) and the lacunosomolecular layer (m). Staining of the dendritic tree of the pyramidal cells and of a capillary. $\times 530$

FIG. 5. Reaction of glial cells in the stratum oriens of the hippocampus. Note the contact with capillaries. $\times 320$

FIG. 6. Control reaction with rabbit normal serum. No specific staining occurs. $\times 200$

localization and is also in agreement with the proposed association of PKA with a microtubule associated protein (MAP 2), which mainly occurs in dendrites (4, 10, 12).

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