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HISTOCHEMISTRY OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE IN NERVOUS TISSUE I. ENZYMEHISTOCHEMICAL INVESTIGATIONS

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Cyclic nucleotide phosphodiesterase was demonstrated in rat and hamster hippocampus by a histochemical method. This method is based on a cerium precipitation technique. The enzymatic reaction was specific and demonstrated the distribution of cyclic nucleotide phosphodiesterase in the hippocampal fields of rats and hamsters. There were no differences between cAMP and cGMP as substrates. There were very strong reaction in the pyramidal layer, in the neuropil of the molecular layer and in the mossy fibre layer. Strong enzymatic activity was localized in pyramidal cells in their dendrites, glial cells and capillaries.

Because of the extraordinary metabolic importance of cyclic nucleotides many histochemical investigations about the building enzymes, adenylate cyclase and guanylate cyclase, were carried out (6). Fewer histochemical studies are available concerning cyclic nucleotide degradating enzymes, cyclic nucleotide phosphodiesterases. The reason seems to be of a methodological nature. First Shanta *et al.* (8) developed a histochemical procedure to localize PDE. This method was used by others with several modifications (1, 2, 3, 8, 11). Our investigations with these methods showed that unspecific reactions in nervous tissue occured. Furthermore, the light and electron microscopical results were not in agreement. So we modified the method further to obtain more specific localizations. We used cerium ions instead of lead for the precipitation of the liberated phosphate ions. The localization of PDE-activity became more specific and all control reactions were negative. The principle of the reaction is the splitting of cyclic nucleotides by PDE and a further degradation of the nucleoside monophosphate by a 5′-nucleotidase. The liberated phosphate ions are precipitated by cerium ions. The 5′-nucleotidase is of an exogenous source.

MATERIAL AND METHODS

Adult albino rats or hamsters were perfused for 10 min with 2% glutaraldehyde

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Abbreviations: PDE: cyclic nucleotide phosphodiesterase.

in 50 mM cacodylate/HCl buffer, pH 7.4, containing 8% sucrose. After decapitation the hippocampi were removed and sectioned with an Oxford vibratome into 80 μm slices. The slices were washed for 2 hr and preincubated 30 min at 4°C in a medium consisting of: 80 mM Tris/maleate buffer, pH 7.5, 2 mM $MgCl_2$, 3 mg/ml snake venom (Crotalus atrox, Sigma), 0.02% Triton X-100. The incubation was carried out 45 min at 37°C in the following medium: 80 mM Tris/maleate buffer, pH 7.5, 2 mM $MgCl_2$, 3 mM cAMP or cGMP, 3 mM $CeCl_3$, 1.5 mg/ml snake venom, 0.02% Triton X-100. The postincubation treatment of the slices was as follows: 3×5 min rinse in incubation buffer, 1×5 min rinse with 5 mM lead citrate, 3×5 min rinse with incubation buffer, 2 min visualization with 2% Na_2S, 3×5 min rinse in distilled water. The slices were then mounted on glass slides, dehydrated and embedded in Entellan.

The lead method was carried out according to Florendo *et al.* (3). For control reactions the snake venom was ommitted or the PDE was inhibited with 3′-isobutyl-1′-methylxanthine.

RESULTS

With the lead method according to Florendo *et al.* (3) nearly all nerval structures reacted (Fig. 1), but the control without snake venom was also stained (Fig. 2). The cerium method (Figs. 3, 4, 5) showed a more distinct reaction and was completely inhibited by 5 mM isobutyl-methylxanthine (Fig. 8). Ommitting the snake venom only little staining was visible in rat hippocampus (Fig. 6) and some more in hamster hippocampus (Fig. 7). No differences were detected using either cAMP or cGMP as substrate (Figs. 3, 4). Higher magnification of the pyramidal layer shows PDE-activity in pyramidal cells and their dendrites (Fig. 9). There were strong reactions in the neuropil of the molecular layer and mossy fibre layer. Capillaries (Fig. 10) and glial cells (Fig. 11) were also PDE-active. A comparison between the reaction for 5′-nucleotidase (not shown) and a PDE-control without snake venom showed the same distribution of reaction product. The addition of NaF inhibited these activities.

DISCUSSION

The use of a lead precipitation technique for demonstration of PDE-activity gave no satisfying results. The cerium technique gives a more distinct reaction, which is completely inhibited by methylxanthines (Fig. 8), an inhibitor of PDE and without influence on 5′-nucleotidase (7). The activity after ommitting the snake venom (Figs. 6, 7) could be a reaction of endogenous 5′-nucleotidase with nucleoside monophosphate liberated by the PDE-reaction. Ueno *et al.* (11) used only endogenous 5′-nucleotidase for PDE demonstration. This is possible in tissues with strong colocalization of PDE and 5′-nucleotidase, but one would never demonstrate soluble isoenzymes of PDE. Therefore we used the snake venom of Crotalos atrox as an exogenous source of 5′-nucleotidase. The advantage is an activation of the PDE by snake venoms through proteolysis (4, 5) or through phospholipases (10). The disadvantage is the resulting bad tissue structure caused by the destroying action of the snake venom. No difference could be found using either cAMP or cGMP as substrate. Most isoenzymes of PDE are able to split both nucleotides. The localization of PDE-activity in neurons

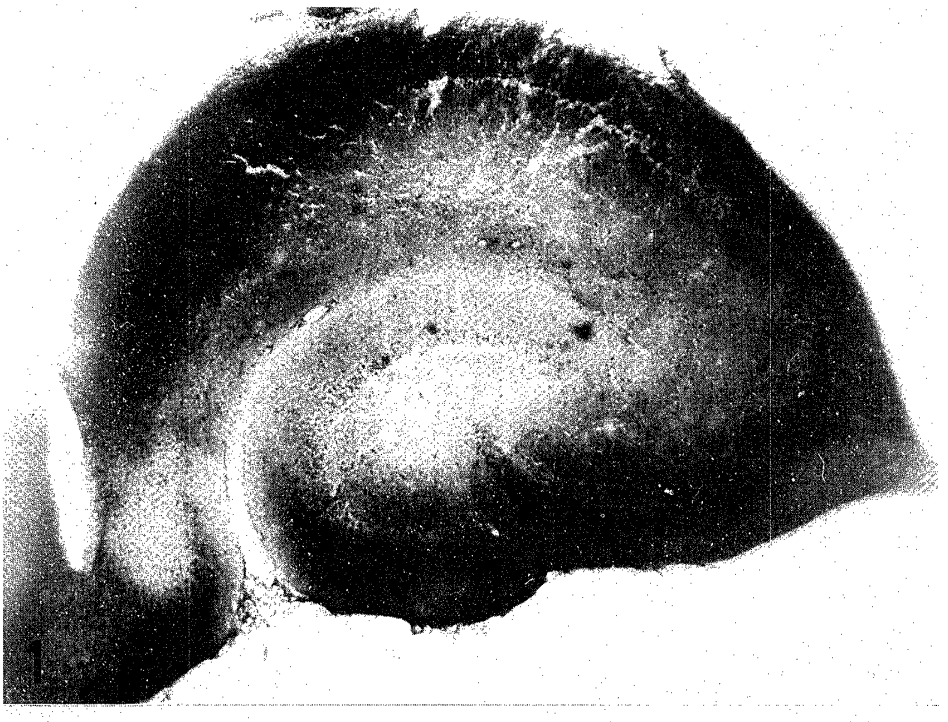

FIG. 1. PDE-reaction in the rat hippocampus according to Florendo *et al.* (3). ×30
FIG. 2. Control to Fig. 1 without snake venom. ×30

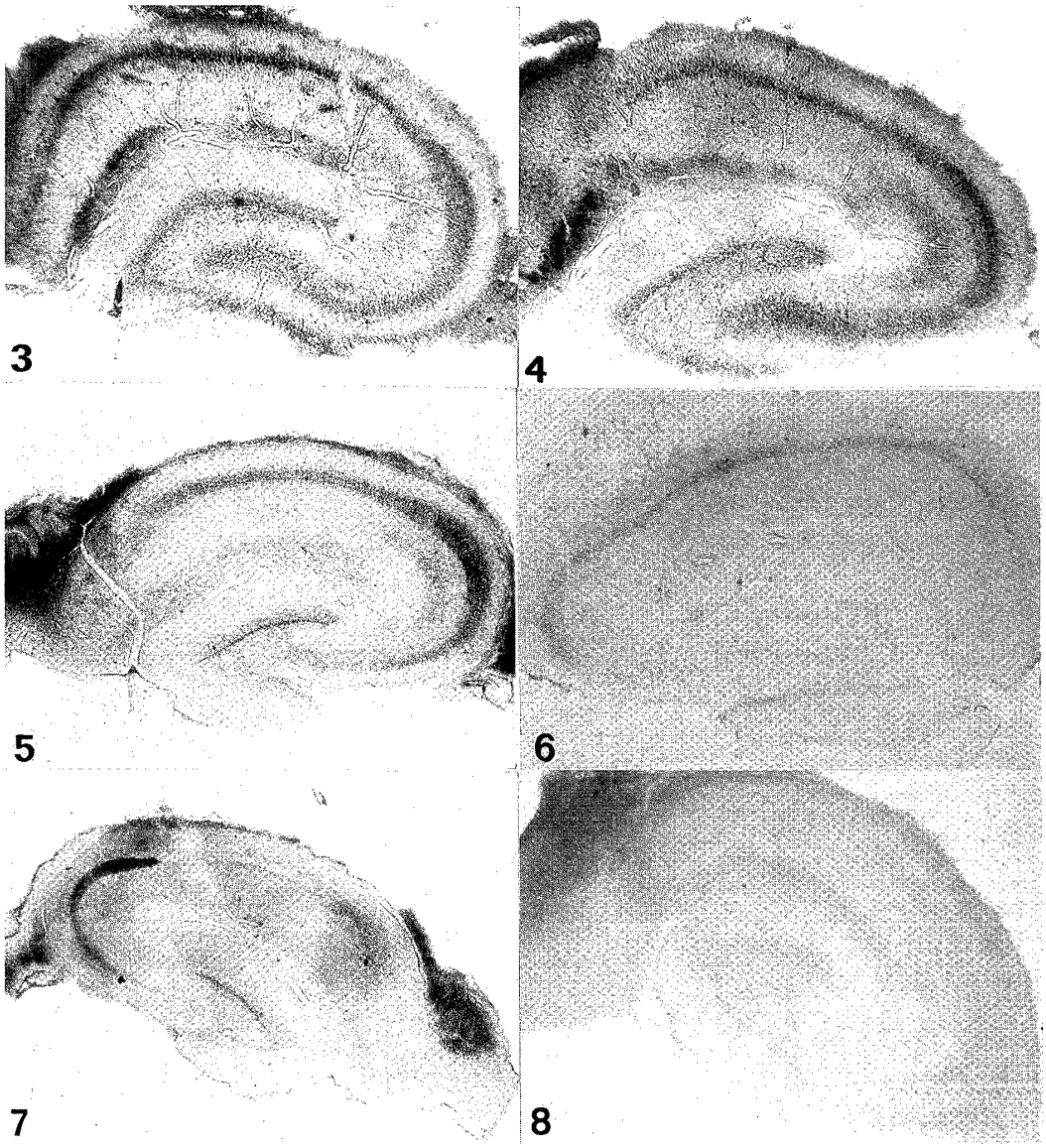

FIG. 3. Localization of PDE-activity in the rat hippocampus with the cerium method, substrate cAMP. ×40

FIG. 4. Like Fig. 3, substrate cGMP. ×40

FIG. 5. Localization of PDE-activity in the hamster hippocampus, cerium method, substrate cAMP. ×40

FIG. 6. Control reaction to Fig. 3, without snake venom. ×40

FIG. 7. Control to Fig. 5, without snake venom. ×35

FIG. 8. Control to Fig. 3, inhibition of PDE with 5 mM 3′-isobutyl-1′-methylxanthine. ×35

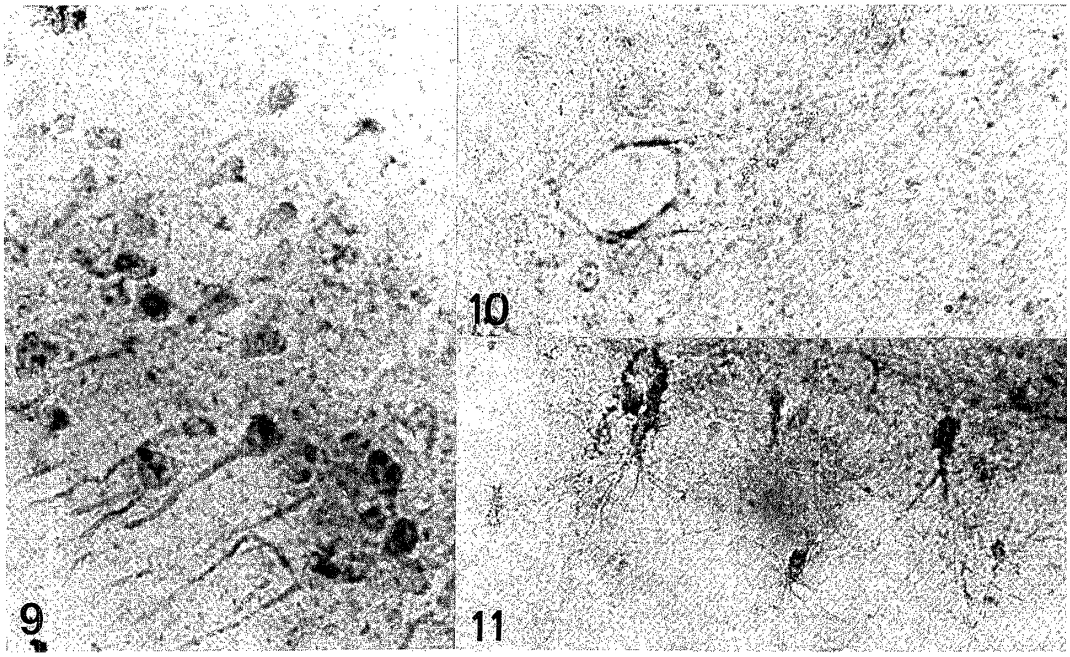

FIG. 9. PDE-reaction of pyramidal cells in the CAl-field of hamster hippocampus, substrate cGMP. ×590

FIG. 10. PDE-reaction in a capillary, substrate cAMP. ×670

FIG. 11. PDE-reaction in glial cells in the white matter of the corpus callosum, substrate cAMP. ×640

(Fig. 9) and glial cells (Fig. 11) should be expected because of the presence of cyclases in these cells. The strong reaction in the neuropil could reflect a reaction in synapses (1-3, 9) and cell processes of glial cells (8) or neurons. Investigations on the electron microscopic level could help to clarify the question, if the reaction in capillaries (Fig. 10) is a reaction in the endothelial cell or in glial processes. Because of the bad tissue preservation using enzymehistochemical PDE-methods, immunohistochemical methods with antibodies against PDE could be helpful to overcome these difficulties.

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