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Immunoelectron microscopical localization of the catalytic subunit of cAMP-dependent protein kinases in brain microtubules and neurofilaments

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The catalytic subunit of cAMP-dependent protein kinases was localized in microtubules and neurofilaments by immunogold electron microscopy. In microtubules, the label was similarly distributed as an immunolabel for the microtubule associated protein MAP 2. The neurofilaments showed no reaction with the MAP 2-antiserum. Our results support the suggestion of an in vivo role of cAMP-dependent protein kinases in the regulation of microtubules. In addition, this is the first demonstration that cAMP-dependent protein kinase is associated with neurofilaments.

Neurofilament; Microtubule; cAMP-dependent protein kinase; Immunogold electron microscopy; Rat brain

1. INTRODUCTION

Cyclic AMP-dependent protein kinases have been shown to be involved in learning and memory [1,2]. However, only little is known on the crucial cellular targets of these enzymes in these processes. One attractive hypothesis is that the dynamic structure of the cytoskeleton, which is controlled by phosphorylation/dephosphorylation, is a substrate for functional plasticity of the central nervous system (cf. [3]).

The microtubule associated protein MAP 2 is one of the major in vitro substrates of brain cAMP-dependent protein kineases [4] and a considerable portion of the enzyme co-purifies with MAP 2 [5]. A physiological significance of this type of kinase in microtubule phosphorylation is suggested also from immunocytochemical colocalization of MAP 2 and the regulatory subunit RII of cAMP-dependent protein kinases in neurons [6,7]. An in situ localization of the catalytic subunit C, the enzymatic active subunit of the kinases, in brain microtubules has not been reported yet.

The significance of cAMP in the phosphorylation of neurofilaments is unclear. A protein kinase which copurifies with neurofilament preparations is cAMP-independent and cAMP added to such preparations showed no significant effect on endogenous protein phosphorylation [8–10]. Recent phosphopeptide analyses of in vitro and in vivo phosphorylated neurofilaments [11,12], however, suggest a role of cAMP-dependent protein kinases also in neurofilament phosphorylation. These kinases could originate from the cytosol or from other cellular structures. Previously, it has been reported that neurofilament preparations free of cAMP-dependent protein kinase activity could be phosphorylated in a cAMP-dependent manner by the addition of microtubules purified by cycles of assembly and dissassembly [13]. Since a large number of morphological, biochemical and biophysical data suggests MAP-mediated interactions between neurofilaments and microtubules, one could assume that cAMP-dependent phosphorylation of neurofilaments is mediated by the MAP-associated protein kinase.

In this study, we have performed an immunoelectron microscopical localization of the C-subunit in rat brain slices. Our results show that cAMP-dependent protein kinases are associated with microtubules as well as with neurofilaments.

2. MATERIALS AND METHODS

Antiserum against the C-subunit of cAMP-dependent protein kinases from bovine heart was produced and characterized as described [14]. Anti-MAP 2 serum was purchased from Sigma (Deisenhofen, Germany) and Protein A-gold from Janssen (Beerse, Belgium).

Male Wistar rats (170–220 g) were anaesthetized with pentobarbital (60 mg/kg) and brain tissue fixed by vascular perfusion with 1.25% glutaraldhyde. After dehydration, tissue pieces were embedded in Lowicryl K4M [15]. Immunocytochemistry was performed as described previously [16] with the antisera used at a dilution of 1:2000 (anti-C-subunit) or undiluted (anti-MAP 2). Protein A labelled with 15 nm gold was used at a dilution of 1:10. The immunolabelled thin sections were stained with uranylacetate and lead citrate and observed and photographed in a Zeiss EM 9 S-2 electron microscope.

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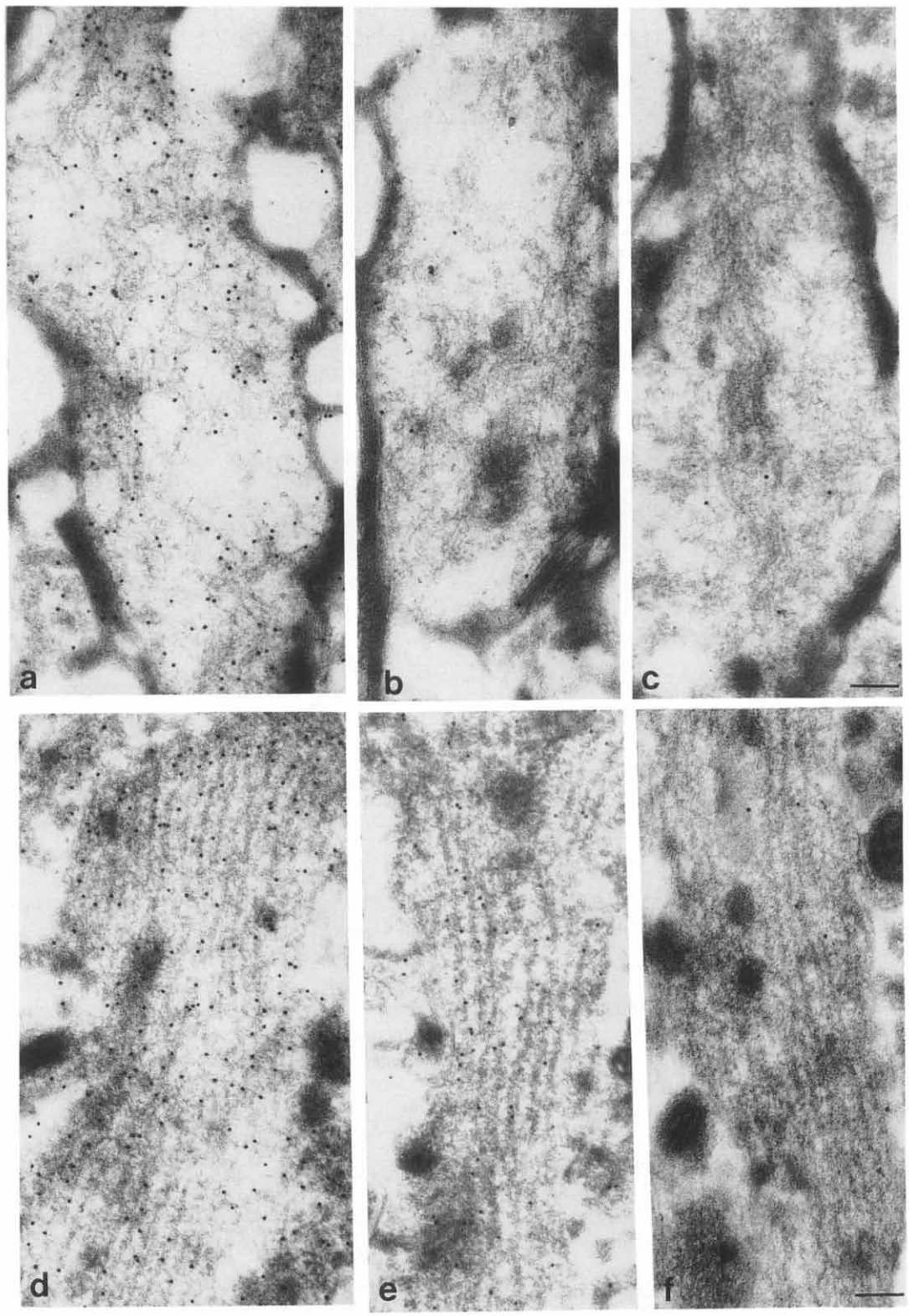

Fig. 1. Immunolocalization of the catalytic subunit of cAMP-dependent protein kinases at neurofilaments (a–c) and microtubules (d–f). (Panels, a,d) Anti-catalytic subunit serum. (Panels b,e) Anti-MAP 2 serum. (Panels c,f) Preimmune serum. Bar = 0.2 μm.

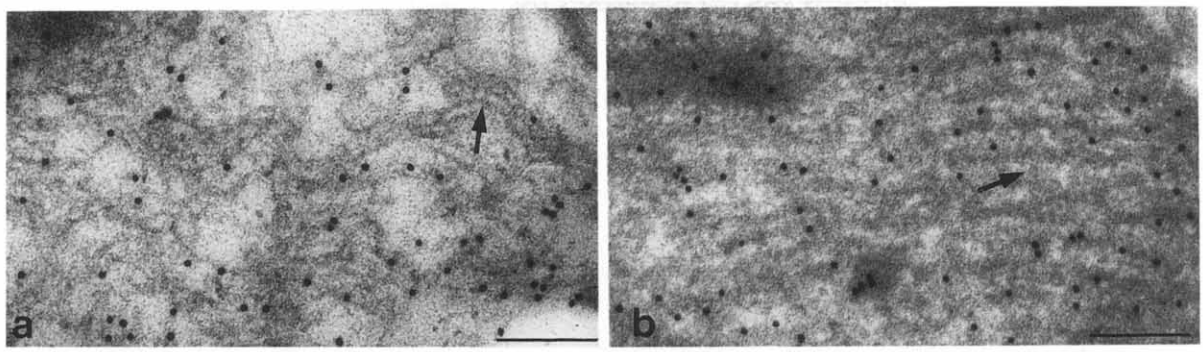

Fig. 2. Details of Fig. 1a (a) and Fig. 1d (b). Bar = 0.2 μm.

3. RESULTS

The immunocytochemical procedure which led to an optimal preservation of the antigenicity of cAMP-dependent protein kinase resulted in some loss in the ultrastructural quality. Nevertheless, microtubules as well as neurofilaments could be clearly identified by their size as well as by their typical forms. Neurofilaments were visible in myelinized axons as relatively thin (diameter 10–15 nm) filaments which were arranged in a characteristic wavy way (Fig. 1a–c; Fig. 2a). Microtubules showed the typical parallel arrangement of larger-sized (diameter 20–30 nm) monomers (Fig. 1d–f; Fig. 2b). Both types of cytoskeletal components showed also typical crossbridges between single elements (Fig. 1; Fig. 2, arrow). Microtubules (Fig. 1e) but not neurofilaments (Fig. 1b) showed an immunoreaction with antiserum against MAP-2. Incubation with the well-characterized [14,16] antiserum against the C-subunit of cAMP-dependent protein kinases led to specific immunolabelling of both, microtubules and neurofilaments (Fig. 1a,d; Fig. 2). In microtubules, the distribution of the label was similar to that of the label for MAP-2 (Fig. 1d,e). In both types of neurofibrils, the majority of gold particles was found at the monomers and not on the crossbridges between them (Fig. 1a,d; Fig. 2). The results were identical with tissues from 3 different rats. Controls, performed by replacement of the antiserum with preimmune serum (Fig. 1c,f), by omission of antiserum, by preincubation of the protein A-gold solution with anti-C serum before use and by preincubation of thin sections with unlabelled protein A before the incubation step with protein A gold (not shown) led to an almost complete abolition of immunolabelling.

4. DISCUSSION

The in situ localization of C-subunit immunoreactivity in microtubules agrees well with previous results of biochemical investigations [5] which suggest that the cAMP-dependent protein kinase is associated with MAP-2. The distribution of the immunolabel is also in accordance with recent results [17] on the sequence of mouse brain MAP 2. Recognition sites for cAMP-dependent phosphorylation are located at the C-terminal part of the molecule which is thought to be clustered near the microtubule binding domain [17].

The presence of C-subunit immunoreactivity in neurofilaments together with the absence of MAP-2-immunoreactivity suggests that neurofilaments can be directly phosphorylated by cAMP-dependent protein kinases and not only by interaction with MAP-2 associated cAMP-dependent protein kinase. Previously, we had immunolocalized the C-subunit also in keratin filaments of parotid gland duct cells, another type of intermediate filaments [16]. Experiments performed in vitro with vimentin [18], desmin [19] and neurofilaments [20] as well as with nerve cell microtubules [21] suggest that the cAMP-dependent protein kinases are involved in filament assembly. The consequences of such phosphorylations for behavioral functions are yet unknown. The present results, however, raise the question whether neurodegenerative diseases like Alzheimer's disease which are characterized by the occurrence of neurofibrillary tangles and by an abnormal distribution and concentration of protein kinase C [22] and casein kinase II [23] in brain cells, have also aberrant cAMP-dependent protein kinases.

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