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High actin concentrations in brain dendritic spines and postsynaptic densities

(neuronal cytoskeleton/immunocytochemistry/dendrites/synaptic junction/synaptic plasticity)

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ABSTRACT **Antibodies against actin were used to corroborate the presence of actin as a major component protein of isolated brain postsynaptic densities. The same antibodies also were used as an immunohistochemical stain to study the distribution of actin in sections of intact brain tissue. This showed two major sites where actin is concentrated: smooth muscle cells around blood vessels and postsynaptic sites. In the postsynaptic area the highest concentration of actin occurs in postsynaptic densities and there also is intense staining in the surrounding cytoplasm, especially within dendritic spines. Antiactin staining was much weaker in other parts of neurons and in glial cells. The high concentration of actin in dendritic spines may be related to shape changes that these structures have been found to undergo in response to prolonged afferent stimulation.**

The postsynaptic membrane of brain synaptic junctions bears a specialized proteinaceous structure known as the postsynaptic density (PSD). Several previous lines of evidence have suggested a special relationship between PSDs and actin filaments. PSDs can be isolated as highly enriched subcellular fractions and when these are extracted with the detergent sodium deoxycholate, an underlying lattice-like cytoskeleton is revealed which consists of filaments of F-actin dimensions (1, 2). Ultrastructural examination of freeze-etched brain samples recently has confirmed the presence of such actin-like filaments closely associated with PSDs in intact tissue and has shown further that these filaments stretch some way into the surrounding cytoplasm (3). A M_r 46,000 polypeptide that comigrates with added muscle actin on $NaDodSO_4$ gels is one of the more prominent constituents of isolated PSDs (4). This component has been shown to be actin by amino acid analysis and reaction with antiserum against actin (5) and by isoelectric focusing and peptide mapping (6). However, it has been pointed out (7) that actin in these isolated brain PSDs could be present as a contaminant—a possibility made more likely by the demonstrated avidity with which PSDs in broken cell preparations bind other filamentous proteins that are not genuine PSD components (8).

One way of corroborating the subcellular affiliation of a given protein in intact tissue is to use immunohistochemical techniques. This approach already has proved useful in demonstrating the association of a variety of proteins—such as tubulin (9), microtubule-associated protein (10), and calmodulin (11)—with PSDs in brain sections and, perhaps more significantly, in showing that other proteins belonging to neighboring structures are not associated with PSDs (8). Antiactin staining of brain sections for light microscopy has suggested an association of actin with synapses (12), but so far this has not been followed up at the ultrastructural level. Such a determination of the ultrastructural distribution of actin is of particular interest because of the variety of roles that have been suggested for synaptic actin. It has been proposed that presynaptically located actin could be involved in exocytotic events leading to neurotransmitter release (13), that actin in the PSD might harness together other postsynaptic molecules such as receptors (2, 5, 6), and it also has been speculated that actin might mediate a physiologically significant contractility of dendritic spines (14).

In this study we have used an antiserum directed against actin to confirm the presence of actin as a major component of isolated PSDs. With immunoperoxidase staining we have demonstrated that within neurons the highest concentrations of actin indeed are associated with PSDs and the immediately surrounding cytoplasm, particularly within dendritic spines.

METHODS

The manufacture and characterization of the antibodies against actin used in this study have been described (15). PSD-enriched subcellular fractions were prepared from rat forebrain (8); their proteins were separated on $NaDodSO_4$-containing polyacrylamide gels (16), blotted onto nitrocellulose sheets (17), and peroxidase stained with antibody exactly as described previously (8). Rat brain tissue was perfusion fixed and immunoperoxidase stained (18) as described (8, 10).

RESULTS

The antibody preparation used here was raised against fish muscle actin and affinity purified on immobilized actin. These antibodies also react with actin in nonmuscle cells (15) and therefore can be used, as we show below, to detect PSD actin which is of the nonmuscle type (6). The selective reaction of these antibodies with actin was evident in various ways. When applied to primary cell cultures from brain, the antibodies stained nonneuronal cells with the typical stress-fiber pattern described in many previous studies. When applied to brain sections, the antibodies stained strongly smooth muscle cells (see below). When tested against nitrocellulose blots of $NaDodSO_4$ gels in which PSD proteins had been separated, they stained the prominent M_r 46,000 band (Fig. 1). The reaction of antiactin with this component is in agreement with previous biochemical and immunochemical evidence (5, 6), indicating that this prominent PSD protein is likely to be actin. The selective reaction of the antibody with this band of actin-like material out of more than 40 bands on a PSD gel blot is consistent with its specificity against actin.

Staining of brain sections with antiactin showed a distinctive pattern in which two cellular elements were reproducibly la-

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Abbreviation: PSD, postsynaptic density.

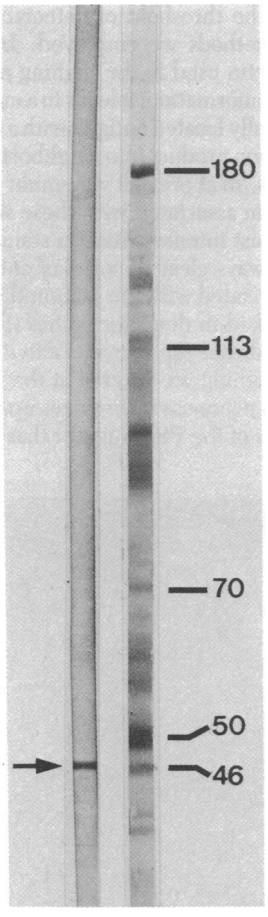

FIG. 1. Reaction of antiactin with PSD proteins. A sample of $NaDodSO_4$-solubilized PSD protein was layered across the top of a slab gel and was electrophoresed so that the separated bands ran continuously from edge to edge. The entire gel was blotted onto a nitrocellulose sheet and this was cut up into 2-mm-wide vertical strips, each thus containing an identical blot of the original gel. Two such blots are shown. The right-hand one is stained for protein with amido black and five of the more prominent PSD polypeptides are indicated with their calculated molecular weights ($M_r \times 10^{-3}$) (for further details, see ref. 8). The band labeled 50 is the M_r 50,000 component that is the major polypeptide of forebrain PSDs (5, 6, 8); the band labeled 46 is the M_r 46,000 actin-like component. The left-hand blot is stained with antiactin and shows the distinct staining of the M_r 46,000 band.

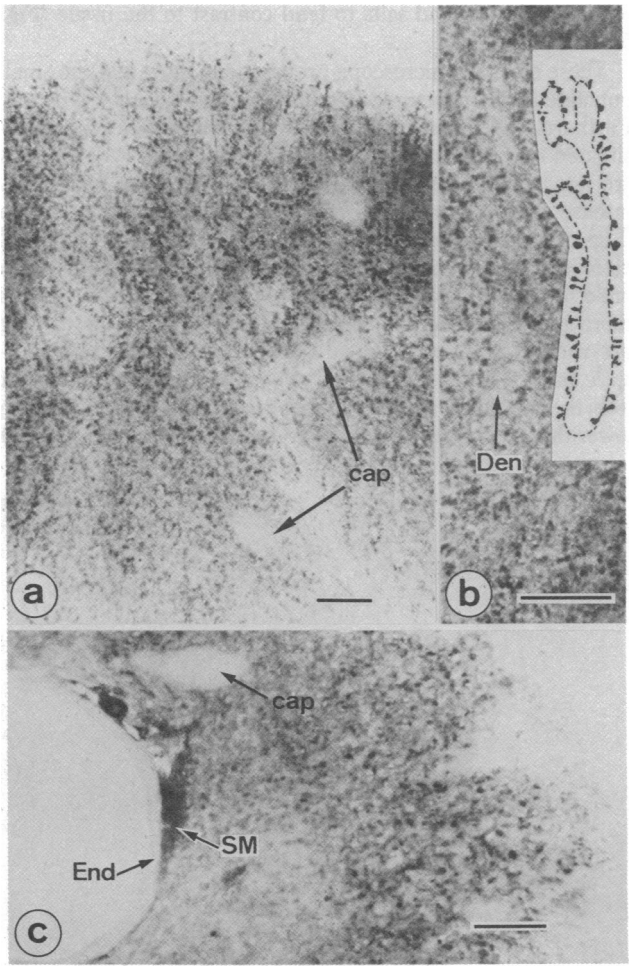

FIG. 2. Brain sections immunoperoxidase stained with antiactin. (*a* and *b*) Vibratome sections of cerebellar cortex; (*c*) a 0.5-μm plastic section of cerebral cortex. Many of the small stained dots that fill the neuropil in *a* are lined up along the surface of dendritic branchlets. This is illustrated at high magnification in *b*, together with a diagram showing the outline of a dendritic segment within the plane of section. There is no staining within the cytoplasm of the dendrite (Den). In *c* a blood vessel (lumen at left-hand border) has a stained smooth muscle cell (SM) closely applied to its unstained endothelial lining (End). There are no stained cells associated with blood capillaries (cap, *a* and *c*). Calibration bars = 10 μm.

beled (Fig. 2). One of these appeared as a myriad of punctate sites, each <1 μm across, filling the neuropil areas of both forebrain and cerebellum (Fig. 2*a*). At the highest light microscopic magnifications it could be seen that these small stained sites were lined up along the surface of sectioned segments of distal dendrites (Fig. 2*b*). The other stained feature consisted of spindle-shaped stained areas that were flattened against blood vessels just inside the endothelial cell layer (Fig. 2*c*). Two cellular compartments exist of this shape and location: smooth muscle cells and astroglial end-feet. However, there are good reasons for believing that this antiactin staining is associated with muscle cells and not glia. First, we only observed it in association with larger blood vessels, never with capillaries. This corresponds to the distribution of smooth muscle cells that are present in the walls of larger blood vessels but are absent from capillaries. On the other hand, astroglial end-feet are present at both sites; hence, if antiactin staining were associated with them, one would expect to see staining alongside blood vessels of all calibers. Second, antiactin staining was never visible in glial cell processes in our preparations, whereas a number of glial cell antigens that we have examined by exactly the same methods as those used here, whether using polyclonal (8, 18) or monoclonal (19) antibodies, gave an easily recognizable distribution pattern of glial processes. Finally, there is the simple expectation that smooth muscle cells should stain strongly with antiactin.

The identity of these stained sites was pursued further in electron microscopic preparations. The vibratome sections on which the light microscopic observations had been made (Fig. 2 *a* and *b*) were postfixed in osmium, embedded in plastic, and sectioned semithin to confirm that the original staining pattern had not been distorted (Fig. 2*c*). Ultrathin sections then were cut from these blocks and examined. The customary heavy metal stains, such as uranyl acetate, were not used because they increase the electron density of the PSD and make the detection of antibody-induced peroxidase stain problematic. On the other hand, though antibody staining can be distinguished easily in such preparations, the tissue is very pale without metal staining (Figs. 3 and 4), and for this reason some sections were stained

on the grid with lead salts to lend contrast to the tissue (Fig. 5).

These electron microscope preparations show that the small punctate antiactin-stained sites in brain neuropil are synaptic. Furthermore, the staining in our preparations is exclusively situated on the postsynaptic side of the synapse where PSDs are intensely labeled and there are substantial, although less intense, deposits in the neighboring postsynaptic cytoplasm (Figs. 3*a* and 4). There is no detectable reaction in the glial cell elements that surround the synapses (e.g., in Fig. 3*a*) nor in endothelial cells that line blood vessels (Fig. 3*b*). In neurons there is no visible staining in cell bodies, nor in axons (including presynaptic terminals), nor in dendrites proximal to postsynaptic areas. This does not necessarily mean that actin is absent from these sites but suggests that its concentration there is much lower than in smooth muscle cells or postsynaptic areas so that it remains below the threshold of detectability of the immunohistochemical methods we employed. Increasing the concentration of antiactin used in the staining procedure does not provide additional information; it leads to a massive overstaining of the postsynaptically located antigen with a consequent spread of peroxidase reaction product into neighboring presynaptic and glial compartments, so at present we cannot say anything about the quantity of actin associated with these sites.

Although the most intense antiactin staining is found in the PSD, there are always clear deposits of antibody-induced reaction product associated with the surrounding cytoplasm. This is particularly marked in dendritic spines (Fig. 4). Although it cannot be excluded that the enzyme activity that gave rise to this cytoplasmic staining was located at the PSD, the extent of the staining and its evenness at distances up to and beyond four times the thickness of the PSD suggest that actin antigen itself

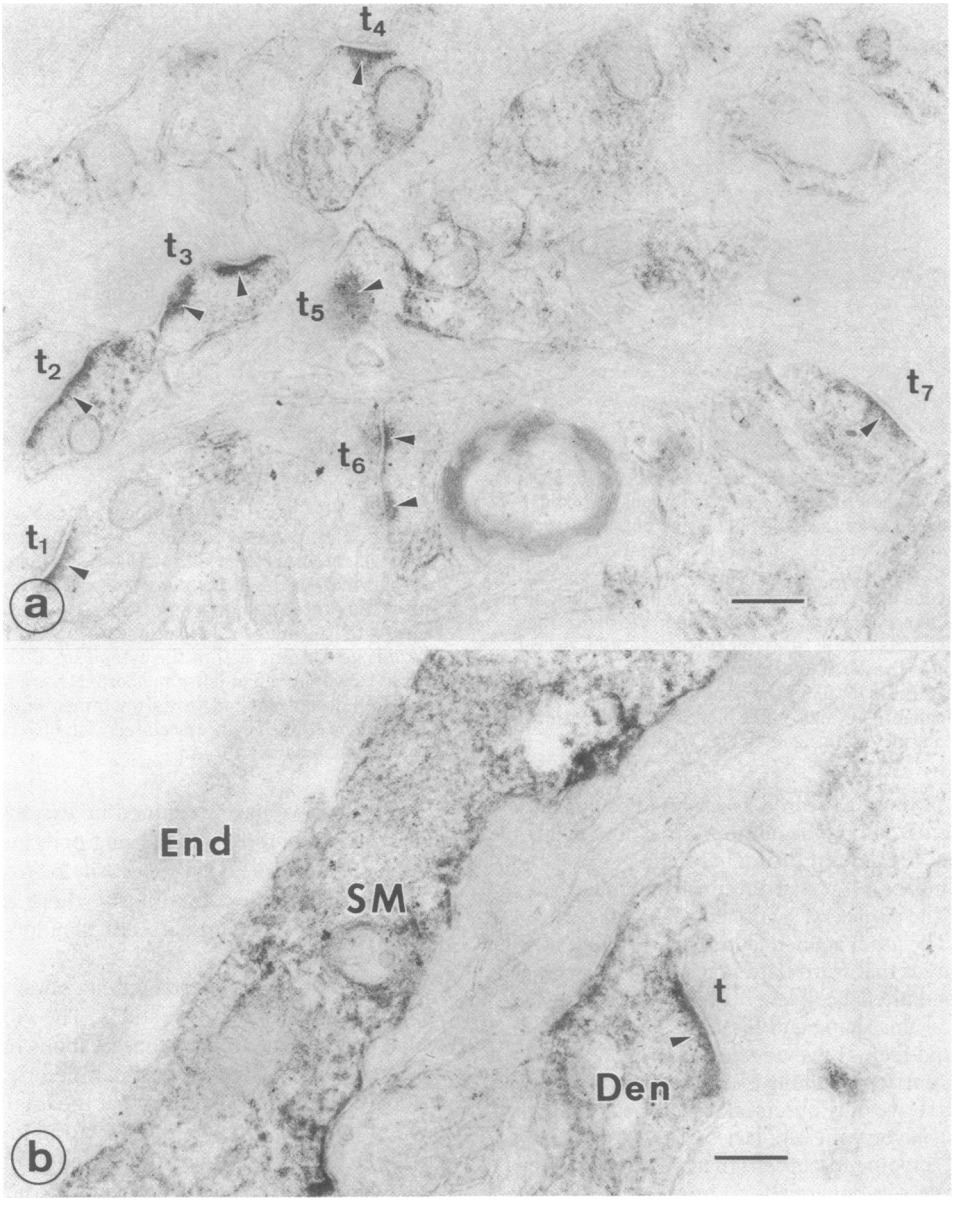

FIG. 3. Electron micrographs of cerebral cortical neuropil stained with antiactin. (*a*) Presynaptic terminals of seven synapses are identified (t_1–t_7). In each of them only the postsynaptic element shows detectable staining that is most concentrated in the PSD (arrowheads). (*b*) A blood vessel (lumen at left) is associated with a stained smooth muscle cell (SM) applied to the lining endothelial cell (End). At a neighboring synapse the presynaptic terminal (t) is unstained, whereas the PSD (arrowhead) is stained strongly as is the cytoplasm of the local area of the dendrite (Den). Calibration bars = 0.5 μm.

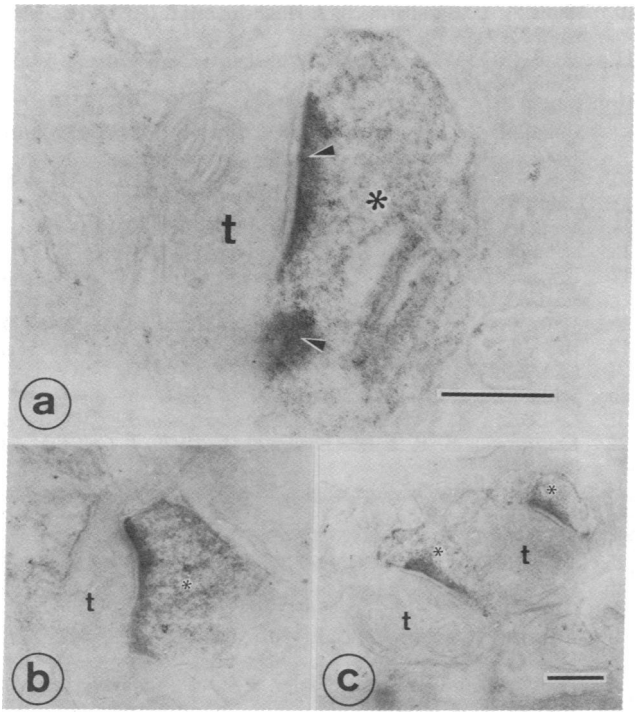

FIG. 4. Examples of synapse onto dendritic spines in the cerebral cortex. In each case, the presynaptic terminal (t) is unstained compared with the postsynaptic spine (identified by asterisks) which is filled with antibody-induced peroxidase reaction product. Staining is always most intense in the PSD (arrowheads in *a*). Calibration bars = 0.25 μm.

is present in the postsynaptic cytoplasm. To gain some idea of the relative amount of postsynaptic actin, we looked for synapses close to blood vessels so that we could examine both stained smooth muscle cells and postsynaptic sites within the same field. An example is shown in Fig. 3*b* in which it can be seen that antiactin staining of the cytoplasm in the postsynaptic region is comparable in intensity to that found in the neighboring smooth muscle cell. This similar intensity of staining in smooth muscle cells and in postsynaptic cytoplasm was observed in more than 20 such situations in which the two cell compartments were found in close proximity within the same evenly stained field.

Fig. 5 shows the relationship of this postsynaptic staining to other neuropil elements that can be clearly seen after lead staining. In addition, the examples in Fig. 5 *b* and *c* show that where there are multiple PSDs at the same synapse, the most intense immunostaining occurs on the independent stretches of PSD.

DISCUSSION

The existence of high concentrations of actin antigen at brain PSDs is in agreement with previous demonstrations that isolated PSDs contain actin (4–6) and that actin-like filaments are associated with both isolated PSDs (1, 2) and PSDs in intact brain tissue (3). The new feature to emerge from the immunohistochemical staining reported here is the strikingly high intensity of antiactin staining of PSDs extending to the surrounding cytoplasm with an intensity that is comparable to the level of staining seen in the cytoplasm of neighboring smooth muscle cells. Our results indicate that levels of actin antigen in the presynaptic terminal are, by comparison, very much lower. However, we cannot exclude the possibility that some special circumstances, such as the presence of actin-binding proteins (20), interfere with immunostaining of presynaptic actin. In this respect it should be noted that labeling with heavy meromyosin

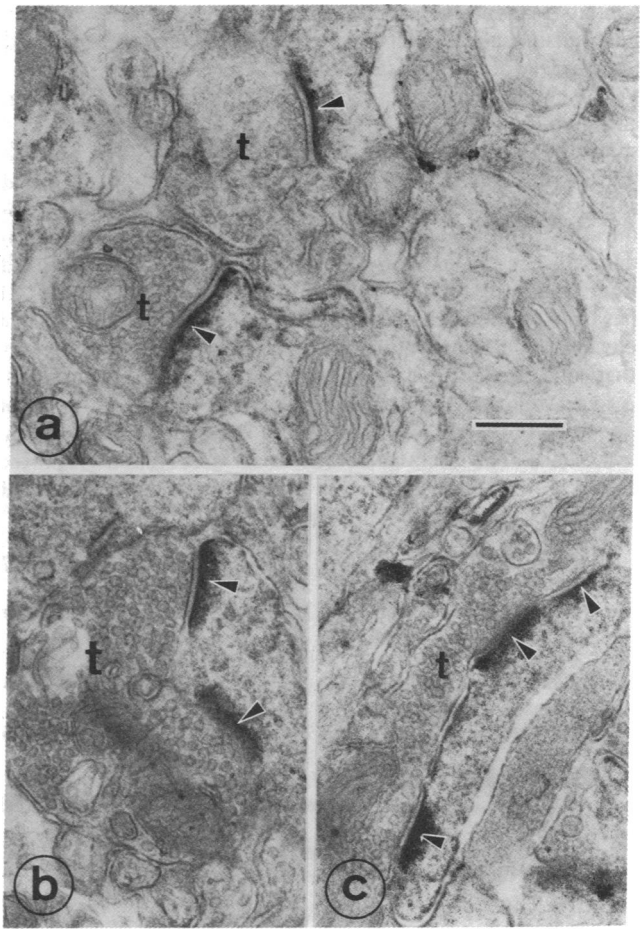

FIG. 5. Micrographs of cerebral cortex stained with antiactin and contrasted with lead citrate. The presynaptic terminals (t) synapse onto dendritic spines within which the PSDs (arrowheads) are distinctly immunoperoxidase stained. Calibration bars = 0.25 μm.

has suggested the presence of actin in both the pre- and postsynaptic components of the synapse (21), although the interpretation of these data does not seem to be straightforward (22). Our finding of high actin concentrations in the postsynaptic area also offers a possible explanation for the disparity, which has been remarked upon (22), between the paucity of visible microfilaments in the cytoplasm of neurons in the brain and the demonstrably high levels of brain actin (23, 24). However, the presence of actin-binding proteins, some of which inhibit the formation of long filaments (20), also could account for the absence of ultrastructurally visible microfilaments outside the postsynaptic area.

Several suggestions have been made already regarding the possible function of postsynaptic actin. The observation that actin-like protein and filaments are concentrated in isolated PSDs led to suggestions that actin filaments may act as part of a cytoskeletal framework for the postsynaptic specialization that would define a synaptic locus and act to harness other molecules, such as neurotransmitter receptors, at their appropriate position on the postsynaptic surface (2, 5, 6). Support for this idea has become available recently from the study of muscle cells in which acetylcholine receptors and actin can be labeled in the same preparation (7). This shows that actin and acetylcholine clusters have remarkably coincident distributions at the endplate, not only in mature cells but, so far as both could be followed, also during development. It is worth noting that the involvement of cytoplasmic actin in marshaling cell surface com-

ponents via transmembrane control is a seemingly common mechanism that has been demonstrated in several cell types and for a variety of antigens (25–28).

The best known role of actin is in mediating cell contraction and motility and in this respect its concentration in dendritic spines at levels apparently similar to those found in smooth muscle cells is of interest. There is considerable evidence that suggests that changes in dendritic spine morphology can be induced by manipulating sensory input (29–33). There also is evidence that shows that alterations in spine morphology can result from tetanic stimulation of inputs to the hippocampus (34). It has been pointed out that these phenomena exhibit the kind of electrophysiologically initiated change in synaptic morphology that could serve as a cellular basis for memory formation (35). A recent reformulation of this idea envisages actin as a mediator of contractile responses of dendritic spines to afferent stimulation (14). The immunohistochemical observations reported here, indicating that actin is concentrated in postsynaptic spines, are consistent with these ideas.

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