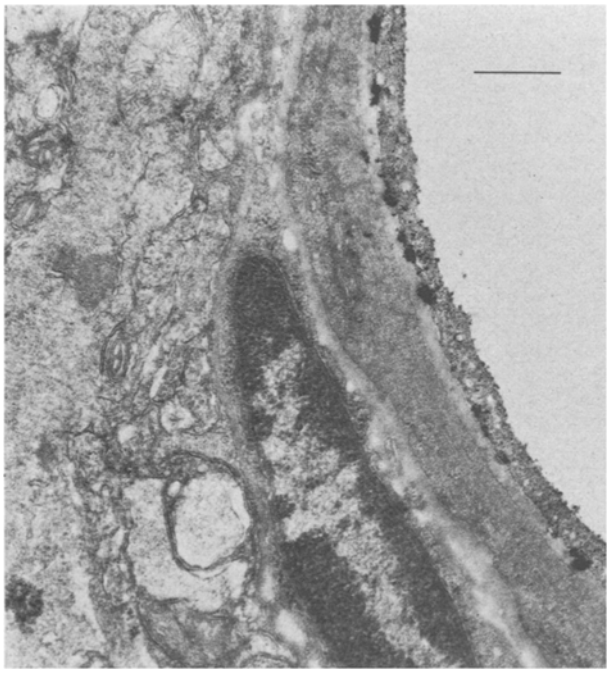

Fig. 1. Incubation in presence of 10 mM K^+. Electron-dense reaction product present on basal surface of endothelial cell. Pericytes and glial processes free of reaction. Bar represents 0.5 μm.

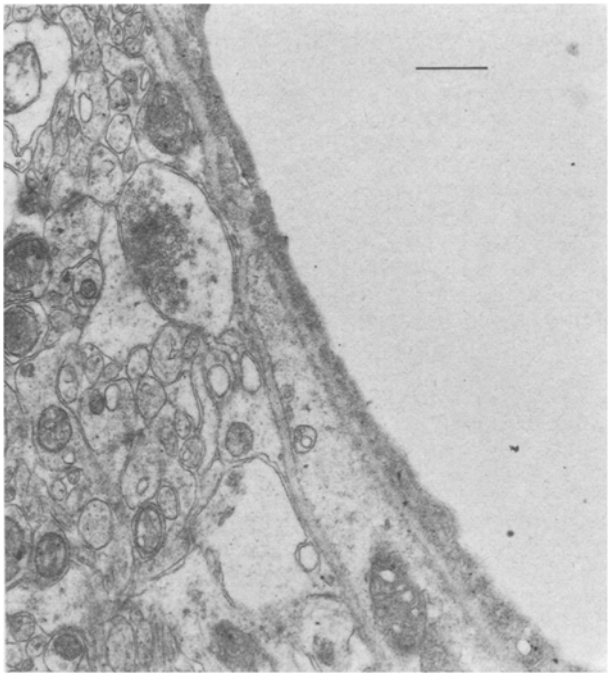

Fig. 2. Incubation in K^+-free medium. Reaction weak or absent in all layers. Bar represents 0.5 μm.

such activity is well preserved in rat brain under the fixation conditions employed here, and is not seriously inhibited by the components of the incubation medium, either singly or together[8]. However, the possibility exists that the endothelial localization is determined by the vascular route of presentation of media rather than by the true anatomical distribution of the enzyme. Presentation of incubation media by perfusion may bias the intensity of reaction so that sites near the vessel lumen appear most active. Unfortunately this is difficult to test as incubation of perfusion-fixed 50 μm slices of brain in vitro gives a widely distributed reaction independent of K^+.

The evidence presented supports the proposal that K^+ concentration in brain intercellular fluid may in part be regulated by active transport through endothelial cells, mediated by Na^+-K^+-ATPase at endothelial plasma membranes. This does not exclude the possibility of glial participation, as the large surface area of the glial processes could compensate for their apparently low Na^+-K^+-ATPase activity per unit area. However, it is probable that K^+ transport at the blood-brain interface depends significantly on the activities of the endothelium, in contrast with the epithelium-dominated transport processes of the choroid plexus[9, 10].

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14-3-2 Protein in rat brain synapses[1]

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Summary. The distribution of the 14-3-2 protein in rat brain synapses was studied by immuno electron microscopy. The protein was localized to the postsynaptic web and to the postsynaptic membrane, but was also prominent both in the presynaptic membrane and in the presynaptic densities. No significant activity was observed in the synaptic vesicles.

The distribution of the 14-3-2 protein, isolated and characterized by Grasso et al.[2], was studied by immunoelectron microscopy. 14-3-2 is a neuronal protein with a mol.wt of about 48,000–50,000[2]. The accumulation of the protein during postnatal development of the mouse and the avian optic tectum has been studied[4, 5], and it was found to accumulate during the first postnatal month of the mouse.

Cicero et al.[6] pointed out a neuronal localization of the 14-3-2 protein by finding a decrease in the content of the protein during nerve degeneration and an increase in the amount during nerve regeneration. Grasso et al.[3] also suggested a synaptosomal localization of the protein by biochemical methods. Due to these results we found it interesting to study the distribution of 14-3-2 by immunoelectron microscopy, and in this paper we have focused our attention on the synaptic region.

Material and methods. The 14-3-2 protein was purified from beef-brain according to Grasso et al.[2, 7], and its antiserum was prepared according to Cicero et al.[6]. The homogeneity of the antigen was controlled by SDS gel electrophoresis (15% polyacrylamide) according to

Shapiro et al.[8]. No impurities were detectable. The antibodies were conjugated to horseradish peroxidase (Sigma Chem. Co., St. Louis, Mo., USA, Type VI) according to Haglid et al.[9]. The labelled antibodies were separated from free aldehyde and free peroxidase on a G-75 sephadex column (Pharmacia, Uppsala, Sweden). Antiserum to 14-3-2, repeatedly absorbed with pure antigen[10], was used as a control.

Rats of the Sprague-Dawley strain, weighing 150–200 g, were anaesthesized with ether and trans-cardially perfused with 4% formaldehyde and 0.1% picric acid in 0.15 M cacodylate buffer, followed by immersion in the same fixative for 1 h. Parts of the CNS were dissected and immersed in 0.15 M cacodylate buffer, pH 7.2. Selected tissue pieces were sectioned in 10–20 μm thick sections on a Vibratome set (Oxford Lab., Calif., USA). The sections were incubated for 45 min in 0.15 M cacodylate-buffer, containing antiserum against 14-3-2 protein coupled with peroxidase. They were then extensively rinsed, and incubated in a cacodylate-buffered solution of 3, 3′-diaminobenzidine and hydrogen peroxide[11]. The sections were rinsed in buffer, postfixed in osmium tetroxide, dehydrated in a graded series of ethanol and embedded in Epon. Thick sections were cut on an ultramicrotome (LKB Ultrotome III) and examined in a phase contrast microscope. Selected areas were sectioned for study in an electron microscope (Siemens Elmiskop 1A). They were studied unstained.

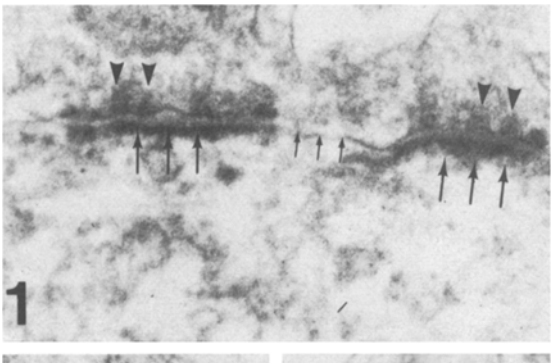
Results and discussion. Moderate peroxidase activity, indicating the presence of 14-3-2 protein, was observed in the neuronal endoplasmic reticular and nuclear membranes. Neuronal plasmalemma showed a low anti-14-3-2 activity. No anti-14-3-2 activity was observed in the Golgi membranes. Moderate peroxidase activity was found in the outer mitochondrial membrane. The latter activity was, however, to some extent also observed in the control sections, and probably due to endogeneous oxidases. Thus, at least part of the mitochondrial peroxidase activity was unspecific and not related to the 14-3-2 protein.

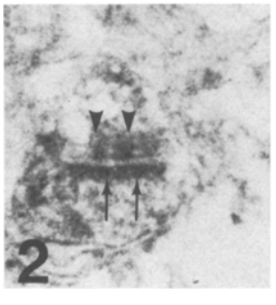
An intense anti-14-3-2 activity was observed in most synapses studied. This synaptic anti-14-3-2 activity was high in the postsynaptic web and the postsynaptic membrane, but was also prominent both in the presynaptic membrane and in the presynaptic densities (figures 1–3). No significant activity was observed in the synaptic vesicles.

Astrocytes, oligodendrocytes, endothelial cells and pericytes all lacked significant activity. No judgement could be made on any activity in myelin, because the endogeneous electron opacity of myelin was considerable even in the control sections.

Cicero et al.[5] suggested a neuronal, especially axoplasmic localization for the 14-3-2 protein. This was confirmed by Grasso et al.[3,7], who presented both biochemical and immunoelectron microscopical data, pointing to a synaptosomal localization of the protein. In this study it is confirmed by immunoelectron microscopy that the 14-3-2 protein is localized to nerve cell processes, including both axons and dendrites, as well as to synaptic junctions in brain. We have shown that the protein is accumulated not only to the postsynaptic membrane, but also to the presynaptic densities, although with a lower anti-14-3-2 activity at this location. This means that there might be a structural and functional similarity in the pre- and postsynaptic membranes concerning the 14-3-2 protein.

The function of the protein is yet unknown, although enolase activity has been found in immunoprecipitation lines in agarose, caused by anti-beef 14-3-2 rabbit serum and brain extracts[12]. However, with this synaptic localization, it is tempting to postulate that the function of 14-3-2 is related to the differentiated functions of neurons.





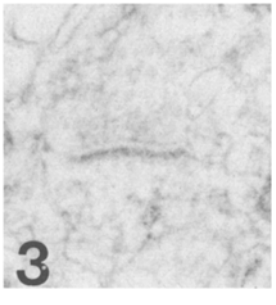


Fig. 1. Synapses in the rat frontal lobe, incubated to show the 14-3-2 protein. Intense anti-14-3-2 activity is observed in the postsynaptic web (large arrow) and in the presynaptic densities (arrow head). Low anti-14-3-2 activity is observed in the neuronal plasmalemma (small arrow). Formaldehyde-picric acid fixation. Unstained. ×45,000.

Fig. 2. Synapses in the rat frontal lobe, incubated to show the 14-3-2 protein. Intense anti-14-3-2 activity is observed in the postsynaptic web (arrow) and in the presynaptic densities (arrow head). Formaldehyde-picric acid fixation. Unstained. ×45,000.

Fig. 3. Synapses in the rat frontal lobe, incubated with antiserum to 14-3-2 which had been repeatedly absorbed with pure 14-3-2. No specific anti-14-3-2 activity is observed. Formaldehyde-picric acid fixation. Unstained. ×45,000.

1 Acknowledgments. Our sincere thanks to Mrs Ulla Svedin, Eng., for skilled technical assistance. This work was supported by grants from the Swedish Medical Research Council, the Medical Faculty of Göteborg, Tore Nilson's Foundation for Medical Research and by the Swedish-Italian Group of Neurobiology. A.G. received financial support from Consiglio Nazionale delle Ricerche (CNR, ROME).

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