Phosphoprotein B-50 and phosphoinositides in brain synaptic plasma membranes: a possible feedback relationship

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In the last decade research on the role of phosphoproteins in neuronal function has yielded evidence that suggests a role for certain phosphoproteins in receptor-activation, receptor-mediated transmembrane signal transduction, ion conductance, etc. At first, attention was focused on cyclic AMP-sensitive protein kinases, and their associated proteins, leading to the identification of an neuron-specific presynaptic protein called synapsin I (Rodnight, 1982; Nestler & Greengard, 1983). In parallel, interest was directed at Ca++/calmodulin and Ca++/lipid-sensitive kinases and their substrates present in the synaptic region (Rodnight, 1983). One such line of research led to the identification and characterization of the synaptic protein B-50 (M, 48,000, pH14.5; Zwiers et al., 1980a). It is likely that the B-50 protein is identical to protein band g5 (Gower & Rodnight, 1982), F1 (Routtenberg, 1982), 47 K (Hershkowitz et al., 1982) or P45p(Ca)(Mahler et al., 1982). In this paper, our present understanding of the role of this protein in synaptic function is reviewed.

Localization of B-50 protein

Using two-dimensional separation techniques and anti-B-50 antisera, the B-50 protein could be detected only in the particulate fractions of brain homogenate and not in subcellular fractions of other rat tissues studied (Kristjansson et al., 1982). Immunostaining of B-50-like proteins in brain homogenates of various vertebrate species revealed the presence of B-50 in human, rat, mouse, hamster, rabbit, cow and chick brain. No B-50 immunoreactivity was obtained in homogenates from Xenopus, goldfish and trout brain (Oesteriicher et al., 1984).

Although endogenous B-50-phosphorylating activity was detected throughout the rat brain, a clear regional activity was obtained. The order of decreasing activity was septum > hippocampus and neocortex > thalamus > cerebellum > medulla oblongata > spinal cord (Kristjansson et al., 1982). Recently, a radioimmunoassay for the B-50 protein has been developed using phosphorylated B-50 as tracer and affinity-purified anti-B-50 immunoglobulins. It was found that synaptic plasma membranes from total rat brain contained 10 µg of B-50/mg of protein and regional studies confirmed the pattern previously reported for the endogenous B-50 phosphorylation activity in brain (septum > hippocampus or cortex > cerebellum; Oesteriicher et al., 1983, 1985).

Immunohistochemical studies of various rat brain areas at the light microscopic level revealed dense immunostaining of B-50 in regions rich in synaptic contacts, whereas white matter and cell perikarya were virtually unstained (Oesteriicher et al., 1981). Recently, we studied the ultrastructural localization of B-50 in ultrathin cryosections of fixed hippocampal tissue and synapto-somes by means of highly specific anti-B-50 immunoglobulins and the protein A-gold staining procedure. Immunoreactivity for B-50 was present exclusively at presynaptic sites of nerve terminals in situ, presumably in synaptic vesicles and closely associated with the inner face of the presynaptic plasma membrane. Not all synapto-somes stained positively for B-50, pointing to a possible association with some, rather than all, trans-mitter systems (Gispens et al., 1985a). Using subcellular fractionation techniques followed by endogenous
phosphorylation of B-50, Sörensen et al. (1981) also concluded that the B-50/B-50 kinase complex is associated with the presynaptic membrane.

**B-50 and the activity of phosphatidylinositol 4-phosphate kinase**

The endogenous B-50-phosphorylating activity was solubilized from the synaptic plasma membranes and enriched by ion-exchange chromatography and ammonium sulphate precipitation. In the precipitate formed between 55% and 80% saturation (ASP50, 80%) endogenous phosphorylation using [γ-32P]ATP labelled only the B-50 protein (Zwiers et al., 1980a). Subsequent studies demonstrated the presence of proteases as well as phosphatidylinositol 4-phosphate (PtdIns4P) kinase activity (Jolles et al., 1980; Zwiers et al., 1980b). In a number of chromatographic steps the B-50 protein kinase/B-50 lipid kinase activity were inseparable; consequently it was assumed that they were part of a multifunctional enzyme complex.

Studies on the significance of such a complex revealed that the degree of phosphorylation of B-50 in the ASP50 fraction, as well as in a lysed crude mitochondrial/synaptosomal fraction, were inversely related to the content of 45000 protein as PtdIns4P kinase (van Dongen et al., 1983). To investigate the modulation of this PtdIns4P kinase activity by the degree of phosphorylation of B-50 in a more direct manner, partially purified PtdIns4P kinase extracts from rat brain were studied in the presence of added purified B-50 preparations which differ in their degree of phosphorylation. In this reconstituted system, conditions could be found where phospho-B-50 (pB-50) reduced PtdIns4P kinase activity and B-50 did not (Zwiers et al., 1985; van Dongen et al., 1985). Since the B-50 protein has no detectable protein or lipid kinase/phosphatase activity itself, it has been suggested that the protein B-50 may be a modulator of PtdIns4P kinase in the presynaptic plasma membrane of rat brain (Gispen et al., 1985b).

**B-50 kinase and protein kinase C**

During studies on the regulation of the phosphorylation of B-50, it was found that the relevant kinase was insensitive to cyclic AMP and cyclic GMP, but was inhibited by the presence of ACTH and stimulated by Ca2+, whereas most likely calmodulin does not act as transducer of the Ca2+ ions to activate the kinase (Gispen et al., 1979; Rodnight, 1982; Sörensen & Mahler, 1983). Extensive studies were carried out to compare the characteristics of B-50 kinase with that of the well-known cyclic nucleotide-insensitive, Ca2+ - and phospholipid-sensitive protein kinase C, present both in brain cytosol and membranes (Inoue et al., 1977; Kuo et al., 1980; Kikkawa et al., 1982).

In view of the similarities in apparent M, isoelectric point, substrate specificity, metal requirements, sensitivity to modulators, phospholipids and protease treatment, Aloyo et al. (1982, 1983) concluded that B-50 protein kinase is very similar if not identical to protein kinase C. These findings link B-50 via its B-50 kinase/protein kinase C to the rapidly growing interest in the role that diacylglycerol (DG) plays in membrane function.

**B-50 as a feedback modulator in PtdIns4,5P2 hydrolysis: functional implications**

From a variety of evidence it has been suggested that the specific hydrolysis of PtdIns4,5P2, inositol trisphosphate (InsP3) and DG is a key event in transmembrane signal transduction and intracellular Ca2+ mobilization. The InsP3 is thought to mobilize Ca2+ from internal stores other than mitochondria (Berridge & Irvine, 1984), whereas DG is known to activate protein kinase C (Nishizuka, 1984). Although in cells and tissues other than brain some other substrate proteins have been identified, presently the function of protein kinase C substrates is largely unknown. In Fig. 1, a model is presented that could account for the related events in synaptic protein and lipid metabolism, allowing B-50 to exert control by feedback inhibition of PtdIns4,5P2 turnover. The validity of this model is presently a subject of further study in our laboratory. Several implications of this model are evident. First of all, the model predicts that in receptor-activated PtdIns4,5P2 hydrolysis, the DG-stimulated protein kinase C will increase the degree of phosphorylation of B-50 thereby decreasing the activity of PtdIns4P kinase necessary to restore PtdIns4,5P2 available for hydrolysis. Recent observations seem to question the primacy of Ca2+ in secretory control (see Baker, 1984). In fact, many secretory systems can be...
activated by agents that promote the breakdown of (polyphosphoinositides, often with little change in intracellular free Ca"²⁺. Furthermore, it has been shown that DG can induce structural transitions in phospholipid bilayers that are crucial to the necessary membrane function in vesicular exocytosis (Das & Rand, 1984). Hence, the B-50 protein may be involved in the control of exocytosis from these presynaptic terminals in which it is localized. In this respect it should be noted that neuropeptides have been shown to act presynaptically as modulators of neurotransmitter release (Versteeg, 1980; Mulder et al., 1984; D. H. G. Versteeg & A. H. Mulder, unpublished work). Previously, it was shown that ACTH and congers inhibit the phosphorylation of B-50 in rat brain synaptic plasma membranes (Zwiers et al., 1978; Ginspen & Zwiers, 1985). The model presented concerning a role for B-50 as a feedback modulator in PtdIns4,5P₂ metabolism, suggests that presynaptic modulation of neurotransmitter release by peptides may be brought about through changes in PtdIns4,5P₂ metabolism (Gisp et al., 1983a,b).

Role of the Golgi complex and characteristics of post-Golgi transport in the biosynthesis of intestinal microvillar proteins

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The small-intestinal enterocyte is a highly polarized cell which possesses a number of enzymes (mostly peptidases and glycosidases) functionally located in the apical (microvillar) portion of the plasma membrane (Kenny & Maroux, 1982). Due to the abundance of the microvillar enzymes, this cell type has become an attractive model system for studying eukaryote plasma membrane protein biogenesis. Work performed in the last few years in several laboratories has revealed a common theme in the biosynthetic events of different microvillar enzymes. This includes a co-translational membrane insertion, high mannose glycosylation of the primary translation product in the rough endoplasmic reticulum and trimming and complex glycosylation of N-linked oligosaccharides (and probably O-linked glycosylation) in the Golgi complex before expression in the microvillar membrane (Danielsen et al., 1984). It has been unclear for some time, however, whether newly synthesized microvillar enzymes, as suggested by Quarioni et al. (1979) and Hauri et al. (1979), pass from the Golgi complex to the basolateral plasma membrane before insertion in the microvillar membrane. A related and more general problem, still unsolved, is at what stage the sorting of newly synthesized microvillar enzymes

Abbreviation used: SDS, sodium dodecyl sulphate.