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Review

Pathophysiological implications of the structural organization of the excitatory synapse

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Abstract

The glutamatergic synapse is the key structure in the development of activity-dependent synaptic plasticity in the central nervous system. The analysis of the complex biochemical mechanisms at the basis of the long-term changes in synaptic efficacy have received a tremendous impulse by the observation that the post-synaptic constituents of the synapse can be separated and purified through a simple procedure involving detergent treatment of synaptosomes and differential centrifugation. In this fraction, called post-synaptic density (PSD), the functional interactions of its constituents are preserved. The various subunits of ionotropic glutamate receptors are held in register with the presynaptic active zone through their interaction with linker proteins. *N*-methyl-D-aspartate (NMDA) subunits NR2A and NR2B, bind to the PSD protein called PSD-95, which in turn binds neuroligins, providing a handle for interacting with neurexin, located in the plasma membrane at the presynaptic active zone. Additional clustering of NMDA receptors is provided through the binding of NR1 subunits to the cytoskeletal protein α-actinin-2. AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate receptors are other important constituents of PSDs and bind to different anchoring proteins. Phosphorylation processes have long been known to modulate NMDA receptor functional activity: the finding that several protein kinases, particularly Ca^{2+}/Calmodulin-dependent protein kinase II and protein tyrosine kinases of the src family, are major constituents of PSDs has allowed to demonstrate that these enzymes are localized in a strategic position of the glutamatergic synapse, so that their activation provides a means for NMDA receptor function regulation upon its activation. The relevance of these mechanisms has been demonstrated in experimental models of pathologies involving deficits in synaptic plasticity, such as in streptozotocin-induced diabetes and in an animal model of prenatal induced ablation of hippocampal neurons. Both animal models display disturbances in long-term potentiation and cognitive deficits, thus providing in vivo models to study pathology related changes in both the structure and the function of the excitatory synapse.

Keywords: Glutamate receptor; Postsynaptic density; Protein kinase; Ca^{2+}/Calmodulin-dependent protein kinase II; Synaptic plasticity; Diabetes; Methylazoxymethanol

1. Introduction

Glutamate is the major excitatory neurotransmitter in the mammalian brain, where it subserves fundamental physiological processes, including activity-dependent synaptic plasticity, and, possibly, the most intriguing of brain functions such as learning and memory. This justifies the great wealth of information gathered in the last few years about the structural organization of the glutamatergic synapse: this understanding is crucial to unravel the complex biochemical mechanisms regulating the functional and structural changes that have been observed as the consequence of preceding synaptic activity in the brain areas mostly involved in learning and memory, i.e., cortex and hippocampus. We will therefore shortly review here the structural features of the glutamatergic synapse, the organization and the complex interplay of its major molecular components with particular reference to protein kinases, the functional significance of this interplay and, finally, its relevance in some experimental pathological conditions involving aberrations in synaptic plasticity and cognitive deficits.

2. Structural features and components of the glutamatergic PSD

Electron microscopists were the first to describe, in the mid 1950s, a prominent thickening of the postsynaptic

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membrane (Palay, 1956), for which the term post-synaptic density (PSD) was coined. The thickness and density of PSDs is variable and falls into two main categories: type I, where the PSD is electron-dense and its size exceeds that of the nerve terminal thickening at the active zone (therefore called asymmetric synapse) and type II, where the PSD is less electron-dense and its size is similar to the presynaptic thickening (therefore termed symmetric synapse) (Colonnier, 1968). Type I synapses are thought to be correlated with excitatory (mostly glutamatergic) transmission, whereas type II mostly with inhibitory transmission utilizing γ-amino-butyric acid (GABA) and glycine (Landis et al., 1974).

The electron density of type I glutamatergic synapses is due to the presence of filamentous and particulate components. Filamentous material are composed by cytoskeletal elements and appear to held together the particulate components (for review, see Ziff, 1997). Isolated PSDs appear as semicircular bands about 400 nm long and 40 nm wide. Interestingly, it has been observed that synaptic activity affects PSD morphology, by inducing its perforation and duplication, indicating that the long lasting biochemical events triggered by glutamate receptor activation entails mechanisms influencing not only signal transmission but also structural remodeling of the synapse (Kennedy, 1993).

Type I PSDs (and from now on the term PSD will be referred only to type I PSDs) can be isolated by treating synaptosomes with detergents such as Triton X-100 to remove membrane lipids and successive centrifugation (Carlin et al., 1980). This subcellular fraction is enriched in structures whose morphology is similar to that observed in intact tissue and has proven to be an invaluable means to study not only the molecular components of the glutamatergic post synaptic organization, but also their interplay from a functional point of view, as outlined below.

There are four major classes of components in PSDs: (i) plasma membrane proteins, (ii) cytoskeletal and neurofilament proteins, (iii) linker proteins and (iv) enzymes and modulatory proteins.

2.1. Plasma membrane proteins

The major components are ionotropic glutamate receptors (iGluRs) including NMDA (Moon et al., 1994), AMPA (Baude et al., 1995) and kainate subunits (Garcia et al., 1998). More controversial is the presence of metabotropic glutamate receptors (mGluRs) (Nusser et al., 1994) and trk A/B neurotrophin receptors (Suen et al., 1997). Less abundant, but functionally relevant among these components, are cell junction proteins such as *N*-cadherin (Beesley et al., 1995), Densin-180 (Kennedy, 1998), neuroligins and β-neurexin (Irie et al., 1997). The major role of these proteins is to keep the PSD in register with the presynaptic active zone. Moreover, the cytoplasmatic C-terminal tail of neuroligins (Irie et al., 1997) binds to PSD-95, one of the major linker proteins (see below).

2.2. Cytoskeletal components

Components are actin, tubulin and a homologue of the neurofilament NF-L subunit (Walsh and Kuruc, 1992). Other so-called cytoskeletal components, such as actin-binding proteins, are better classified as linker proteins, since it has been recently shown that brain spectrin (Wechsler and Teichberg, 1998) and α-actinin-2 (Wyszynski et al., 1997) serve as linkers between actin filaments and NMDA receptor subunits, therefore influencing the correct clustering of ionotropic receptors.

2.3. Linker proteins

The other linker proteins belong to a family of synaptic proteins homologous to the product of the Drosophila gene *disc large* and includes: PSD-95/SAP (Synapse Associated Protein) 90, SAP97, chapsyn-110/PSD-93 and SAP102. This family of proteins is characterized by having three association domains, called PDZ domains in virtue of the fact that similar domains are found in PSD proteins, in Drosophila Discs Large and in ZO-1, an epithelial tight junction protein, an SH3 (src-homology-3) domain and an enzymatically inactive guanylate kinase-like (GK) domain. For this reason, these proteins are also defined as a subclass of the MAGUK (membrane-associated guanylate kinases) superfamily of proteins. The functional significance of some of these domains will be discussed below. Other proteins with PDZ domains are GRIP (glutamate receptor interacting protein) (Dong et al., 1997), and ABP (AMPA receptor binding protein) (Srivastava et al., 1998) with seven and six PDZ domains, respectively. GRIP and ABP lack both the SH3 and the GK domains found in PSD-95. Finally, there are adaptor proteins linking enzymes to PSD: AKAP (A kinase anchoring protein) 79 (Carr et al., 1992) and AKAP 150. AKAP 79 binds also calcineurin (Coghlan et al., 1995) and protein kinase C (Klauch et al., 1996).

2.4. Enzymes and modulators

The last major class of proteins present in PSDs are several enzymatic systems, most of which regulating the phosphorylation state of several PSD substrates: Ca^{2+}/calmodulin-dependent protein kinase type II (Kennedy et al., 1983), non-receptor protein tyrosine kinases of the src family (Suzuki and Okumura, 1995), protein kinase C isozymes β and γ (Suzuki et al., 1993), cAMP-dependent protein kinase (Carr et al., 1992), protein phosphatase 1 (Dosemeci and Reese, 1993) and 2B (calcineurin) (Coghlan et al., 1995), neuronal NO synthase (Brenman et al., 1996), ERK2-type mitogen activated protein kinase (Suzuki et al., 1995) and calpain (Dosemeci and Reese, 1995). Recently two groups (Chen et al., 1998; Kim et al., 1998) have identified a protein belonging to the family of ras-GTPases, named SynGAP, since it is exclusively expressed in synapses of mammalian brain and highly enriched in PSDs. However, this is not the only protein

participating in the ras signaling system, since a putative rho/rac effector protein has also been observed in glutamatergic PSDs and called citron (Zhang et al., 1999). Both SynGAP and citron contain the consensus sequence (see below) that allows their anchoring to PSD-95.

3. Functional interactions between PSD components

This vast array of cytoskeletal and regulatory proteins found in the PSD interact to constitute the glutamatergic postsynaptic signal transduction machinery, coordinating activity-dependent changes in postsynaptic structures, including long-term potentiation and long-term depression, the cellular basis for learning and memory. The picture emerging from studying these interactions is that these molecular components are essential for (i) clustering glutamate receptors just opposite to the presynaptic active zone, (ii) modulating glutamate receptor sensitivity and (iii) inducing long-lasting changes in the preactivated synapse.

3.1. Clustering of glutamate receptors

Essential players for clustering glutamate receptors are PSD-95/SAP90, SAP97, chapsyn-110/PSD-93, SAP102, GRIP and ABP. All these proteins are characterized by having several PDZ domains, as mentioned above. PDZ domains are constituted by about 100 residues with a number of 'Glycine–Leucine–Glycine–Phenylalanine' repeats able to bind specific motifs found in the cytoplasmatic C-terminal region of iGluR subunits (O'Brien et al., 1998).

The NMDA receptor subunits 2A (NR2A) and 2B (NR2B) bind to the first two PDZ domains of PSD-95/SAP90, through the terminal Threonine/Serine-X-Valine-COOH (T/SXV, where X stands for any amino acid residue) motif (Kornau and Scott, 1995). The third PDZ domain in PSD-95/SAP90 binds to neuroligins, who also ends with the T/SXV motif. Since neuroligins bind to β-neurexin, as mentioned above, PSD-95/SAP90 is essential for clustering NMDA receptors at the right spot of the synapse. NMDA receptors are constituted by hetero tetra- (or penta-)mers of NR2A/B and NR1. NR1, in turn, provides a direct link to cytoskeletal proteins through the interaction of the C0 and C1 regions with α-actinin-2 and other two filamentous proteins, respectively. These interactions provide a further means for the clustering of NMDA receptors in PSD. Very recently PSD-95 null mutant mice were obtained (Migaud et al., 1998): surprisingly, these animals showed a normal synaptic localization of NMDA receptors, suggesting that their clustering is not affected by the absence of PSD-95 and strengthening the importance of this NR1 interaction with cytoskeletal proteins. The mutant mice, however, showed a dramatic increase in long-term potentiation, indicating that NR2-PSD-95 interactions play a major role in signal transduction pathways controlling synaptic strength.

AMPA receptors (composed of four subunits GluR1–4) possess a different C-terminus motif: GluR2 and GluR3 have a carboxyl terminal sequence –Glutamate–Serine–Valine–Lysine–Isoleucine. Using a two-hybrid system, GRIP was isolated (Dong et al., 1997) and shown to contain seven PDZ domains. PDZ 4 and 5 probably interact with two GluR2/3 subunits forming the heterotetrameric AMPA receptor. PDZ 1 and 7 seem to be anchored to cytoskeletal elements (O'Brien et al., 1998). GluR1, on the other hand, possesses a Leucine in the terminal position and a Threonine in the C-terminal -2 position: this change in amino acids allows for the association with another member of the SAP proteins, SAP97. This interaction is specific for GluR1, since neither GluR2/3 nor NMDA subunits coimmunoprecipitate with SAP97. ABP seems to be present in at least three isoforms generated by the same gene. ABP shares high homology (64–93%) with GRIP in the PDZ domains and interacts with the GluR2/3 C-terminal Valine–Lysine–Isoleucine–COOH motif via class II hydrophobic–PDZ interactions, distinct from the class I PSD-95–NMDA interaction. Moreover, ABP and GRIP form homo- and hetero-multimers through PDZ–PDZ interactions (Srivastava et al., 1998). Therefore, NMDA and AMPA receptors seem to utilize different strategies for their clustering at the glutamatergic synapse.

Some of the kainate receptor subunits (GluR5–7, KA1 and 2) have shown to be also present in PSD and to coimmunoprecipitate with some of the SAP protein family. GluR6 coprecipitates with PSD-95/SAP90, SAP97 and SAP102, whereas KA2 coprecipitates only with PSD-95/SAP90 and SAP102, but not with SAP97 (Garcia et al., 1998). But whereas GluR6, having the C-terminal motif –Glutamate–Threonine–Methione–Alanine, interacts with PDZ1 of PSD-95/SAP90, KA2 binds to SH3 and the GK domains of the same protein. The SH3 binding is mediated by a C-terminal proline-rich sequence, as predicted. The binding to GK domain seems to involve a different site (yet to be determined) from that occupied by the novel GK anchoring protein, of unknown function (Kim et al., 1997).

Regarding mGluRs, group I (positively coupled to the phosphoinositol cycle) seems to bind to Homer, a 186 amino acid protein found in PSDs and possessing one PDZ domain (Brakeman et al., 1997). Interestingly, PSDs contain also some of the G-proteins, in particular Gi2α, although in quantities which are less than those present in synaptic membranes (Wu et al., 1992). This might be in line with the notion that mGluR (group II and III are negatively coupled to adenylyl cyclase trough a Gi protein) are found on the border of PSDs (Lujan et al., 1997).

3.2. Functional interactions with kinases and other enzymes

The SAP protein family has an other important function, besides binding and clustering iGluRs: they bind

other constituents of PSDs, such as enzymes and modulatory molecules. Indeed, PSD-95/SAP90 binds neuronal NO synthase at its PDZ 2, via a PDZ–PDZ interaction (Brenman et al., 1996). Neuronal NO synthase (a calcium-dependent enzyme) is therefore held in the appropriate position to sense the calcium influx through NMDA channel opening. The third PDZ domain of PSD-95/SAP90 binds SynGAP (Chen et al., 1998; Kim et al., 1998), providing a link between the NMDA receptor and the mitogen-activated protein kinase pathway. A similar link is provided also by the interaction of citron with PSD-95/SAP90, which, as mentioned above, is a putative rho/rac effector protein. Interestingly, SynGAP and citron have opposing effects on the MAP kinase pathway, being inhibitory the first and stimulatory the second. Phosphorylation of SynGAP by Ca^{2+}/Calmodulin-dependent protein kinase II prevents its inhibitory action on mitogen activated protein kinase (Chen et al., 1998; Kim et al., 1998), leading therefore to promotion of transcription in the nucleus (Fig. 1).

Very recently an intriguing finding has been reported by Tezuka et al. (1999). They found that Fyn and other members of the src family (Yes, Src and Lyn) associate with PSD-95 in an unconventional way: the SH2 domain of Fyn, which is found close to the myristoylated N-terminus, binds to a PSD-95 region containing the third PDZ domain. Moreover, this binding, does not require Tyrosine phosphorylation, as normally occurs for SH2 domains.

As mentioned previously cAMP-dependent protein kinase, protein kinase C isozymes and protein phosphatases are tethered to PSD through adaptor proteins of the AKAP family. Whether also these proteins are bound to cytoskeletal elements present in PSDs remains to be demonstrated.

A different anchoring system to PSD seems to operate for Ca^{2+}/Calmodulin-dependent protein kinase II. Coprecipitation experiments suggested that Ca^{2+}/Calmodulin-dependent protein kinase II is associated to NR2, but not AMPA subunits. This association has been further con-

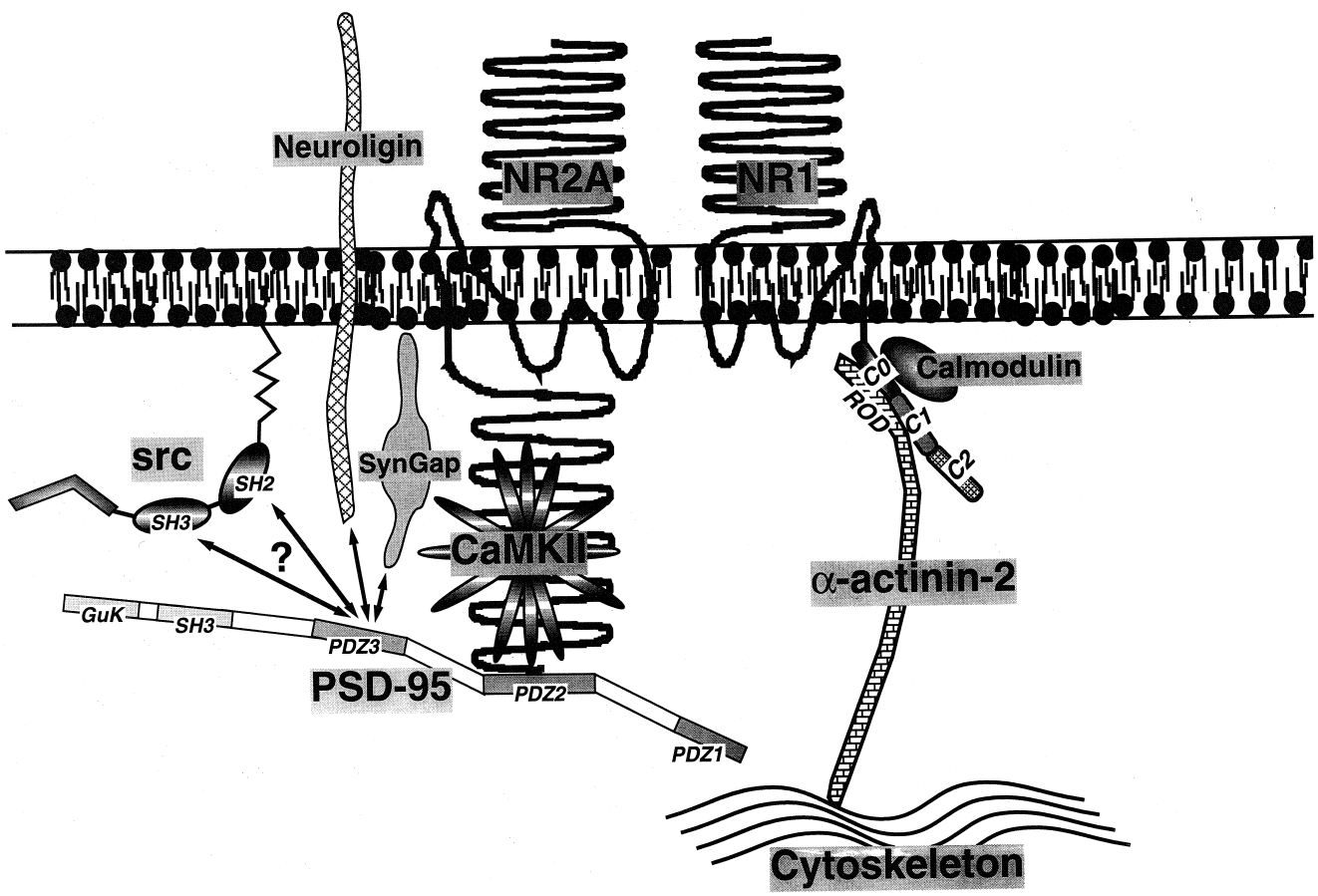

Fig. 1. Schematic representation of PSD proteins associated to NMDA receptor subunits. The clustering of NMDA receptors in PSD is regulated by association to cytoskeletal proteins (α-actinin-2) as well as to the scaffolding protein PSD-95, which is capable of binding several proteins in the PDZ3 domain. The different NMDA subunits are also associated to several transducing molecules (Ca^{2+}/Calmodulin-dependent protein kinase II, src, calmodulin, SynGap), thus providing a direct link between activation of NMDA channel and different intracellular biochemical cascades. Both the structural organization and the regulation of transduction pathways associated to NMDA receptors can be profoundly modified in pathological conditions implying alterations in the plasticity of the excitatory synapse. See text for details.

firmed by overlay and crosslinking experiments (Gardoni et al., 1998). Moreover, it has been shown that the association domain resides in the 1349–1464 C-terminal region of NR2A, the same region binding the PDZ domain of PSD-95/SAP90 (Gardoni et al., submitted). Whether Ca^{2+}/Calmodulin-dependent protein kinase II binding competes and displaces PSD-95/SAP90 binding, as well as whether other transducing enzymes found in PSD (i.e., protein kinase C) might regulate the functional interaction of Ca^{2+}/Calmodulin-dependent protein kinase II to NMDA receptors remains to be determined. Interestingly, autophosphorylated Ca^{2+}/Calmodulin-dependent protein kinase II seems to enhance considerably binding to NR2A (Gardoni et al., submitted), as also very recently reported by Shen and Meyer (1999) in hippocampal neurons transfected with green fluorescent protein-tagged Ca^{2+}/Calmodulin-dependent protein kinase II. The significance of this will be illustrated in the next section.

4. Kinases: key enzymes in the right place

Several lines of evidence suggest a fundamental role of both Ca^{2+}/Calmodulin-dependent protein kinase II and protein tyrosine kinase in activity-dependent changes of the glutamatergic synapse: mice with the knock out for the α-subunit of Ca^{2+}/Calmodulin-dependent protein kinase II show a severe disruption in both long-term potentiation and long-term depression (Silva et al., 1992b) and deficits in the Morris water maze, a test for spatial memory in rodents (Silva et al., 1992a). Similarly, knock out of Fyn impairs certain forms of long-term potentiation and spatial memory (Grant et al., 1992). Biochemical evidence for a direct role of these kinases comes from the observation that NR2A/B subunits of the NMDA receptor are phosphorylated by Ca^{2+}/Calmodulin-dependent protein kinase II and protein tyrosine kinases. Indeed, Ca^{2+}/Calmodulin-dependent protein kinase II phosphorylates NR2 (Fong et al., 1989) but not NR1 subunits, on Serine residues in the large intracellular C-terminal region, being Serine-1303 in NR2B the major site of phosphorylation (Omkumar et al., 1996). This site is equivalent to Serine-1289 in the NR2A subunit (Gardoni et al., submitted). Protein tyrosine kinases also phosphorylate the large C-terminal region of both NR2A and NR2B on an as yet unidentified Tyrosine (Suzuki and Okumura, 1995). Also in this case, there is no evidence of NR1 phosphorylation in vivo. Functionally, both Serine and Tyrosine phosphorylations have been shown, although indirectly, to be of importance on NMDA channel activity. For instance, Yu et al. (1997) demonstrated that activated Src increases NMDA receptor channel activity. As reported previously, Ca^{2+}/Calmodulin-dependent protein kinase II knock out impairs long-term potentiation and long-term depression. Conversely, constitutive activation of Ca^{2+}/Calmodulin-dependent protein kinase II by mutating Threonine-286 with Aspartate (Fong et al., 1989) changes the frequency of extracellular stimulation at which long-term potentiation and long-term depression are induced (Mayford et al., 1995). Surprisingly, as yet there are no direct evidence of a causal relationship between protein tyrosine kinases and/or Ca^{2+}/Calmodulin-dependent protein kinase II activation, phosphorylation of NMDA subunits and changes in ion currents. Despite this, it is fair to assume that Ca^{2+}/Calmodulin-dependent protein kinase II and protein tyrosine kinases play a central role in the activity-dependent enhancement of synaptic efficacy. In this respect, it is interesting to underline that Ca^{2+}/Calmodulin-dependent protein kinase II, being physically associated to NR2A subunits, is in the ideal place to sense calcium entry through the ion channel. When the NMDA channel is opened by agonists, calcium entry activates Ca^{2+}/Calmodulin-dependent protein kinase II which is bound to NR2A subunit. This activation entails autophosphorylation and further association of Ca^{2+}/Calmodulin-dependent protein kinase II to NMDA (Gardoni et al. submitted). Activated Ca^{2+}/Calmodulin-dependent protein kinase II promotes phosphorylation of NR2A/B subunits, hence sustained channel activity. Since also protein tyrosine kinases phosphorylate NR2A/B subunits, but at a different residue, it might well be that these kinases work in concert and that they modulate each other through reciprocal phosphorylation. Work is currently in progress in our laboratory to ascertain this relationship.

Large intracellular domains of NMDA receptor subunits NR1, NR2A and NR2B may interact also with other signal transducing proteins. Using the two-hybrid system, calmodulin was found to interact with the –COOH terminus of the NR1 subunit, inactivating the NMDA channels in a Ca^{2+}-dependent manner (Hisatsune et al., 1997). Furthermore, protein kinase C-mediated phosphorylation on Serine residues of NR1 was shown to decrease NR1 affinity for calmodulin and, thereby, to inhibit the inactivation of NMDA receptors mediated by calmodulin. Moreover, recent studies have demonstrated that mutation of specific calmodulin binding sites in the C0 region of NR1 subunits blocks the Ca^{2+}-dependent inactivation of NMDA channels (Zhang et al., 1998); this inactivating effect of calmodulin can also be prevented by coexpressing a region of α-actinin-2 known to interact with the C0 region of NR1.

5. Ca^{2+}/Calmodulin-dependent protein kinase II and NMDA phosphorylation in experimental models of impaired long-term potentiation and learning tasks

It has been mentioned previously that knock outs for Ca^{2+}/Calmodulin-dependent protein kinase II and protein tyrosine kinases abolish or impair certain forms of long-

term potentiation and long-term depression, that induction of long-term potentiation promotes Ca^{2+}/Calmodulin-dependent protein kinase II activation (Barria et al., 1997) and constitutive activation of Ca^{2+}/Calmodulin-dependent protein kinase II changes the frequencies inducing long-term depression and long-term potentiation (Mayford et al., 1995).

But there is also evidence that pathological changes of the central nervous system involving impairments of cognitive tasks and long-term potentiation have a counterpart in changes of Ca^{2+}/Calmodulin-dependent protein kinase II activity and NMDA receptor phosphorylation.

5.1. Streptozotocin-diabetic rats

Moderate disturbances of learning and memory have been reported as a complication of diabetes mellitus in patients (McCall, 1992; Biessels et al., 1994). In animal models of the diabetic pathology, such as the streptozotocin-diabetic rat, spatial learning impairments have been reported. This cognitive deficit is associated with changes in hippocampal synaptic plasticity, including an impaired expression of long-term potentiation and enhanced expression of long-term depression (Biessels et al., 1998). In situ hybridization histochemistry revealed that the transcript levels of NR1 and NR2A subunits of NMDA receptor were unmodified in rats with a diabetes duration of 3 months when compared to age-matched controls. However, the mRNA levels of NR2B in diabetic rats were reduced when compared to control rats (Di Luca et al., 1999). Indeed semiquantitative analysis of transcripts revealed a decrease in the NR2B mRNA levels in hippocampus of diabetic rats when compared to controls. NR1 and NR2A as well as GluR1, GluR2/3, PSD-95 and Ca^{2+}/Calmodulin-dependent protein kinase II protein concentrations in hippocampal PSD were the same in both control and diabetic rats, whereas the immunoreactivity for NR2B was reduced by about 40%. In addition, the capability of Ca^{2+}/Calmodulin-dependent protein kinase II to phosphorylate exogenous substrates, such as syntide-2, as well as the endogenous phosphorylation of both NR2A and NR2B subunits of NMDA receptor were reduced in hippocampal PSDs of streptozotocin-diabetic rats as compared to controls. Insulin treatment of diabetic rats for a 3-month period after the establishment of diabetes, only partially restored Ca^{2+}/Calmodulin-dependent protein kinase II activity, as well as NR2B levels. Recently it was reported that insulin treatment, nearly normalizing blood glucose levels, body weight and impaired nerve conduction velocity, only partially restored water maze performance and long-term potentiation in streptozotocin-diabetic rats (Biessels et al., 1998). Interestingly, these animals also showed partial restoration of Ca^{2+}/Calmodulin-dependent protein kinase II activity and NR2B levels (Di Luca et al., 1999), strengthening the link between behavioral, electrophysiological and neurochemical parameters.

5.2. Prenatal ablation of hippocampal neurons

To examine the implication of Ca^{2+}/Calmodulin-dependent protein kinase II activity and NMDA receptor phosphorylation in synaptic plasticity, we also used an animal model characterized by developmentally induced targeted neuronal ablation within the cortex and the hippocampus and lack of long-term potentiation (Methylazoxymethanol treated rats) (Cattabeni and Di Luca, 1997). At variance with animal models in which alteration in long-term potentiation is obtained by specific kinase knock-outs (Silva et al., 1992b; Abeliovich et al., 1993), in this model the lack of long-term potentiation is due to a partial prenatal ablation of CA1 neurons. This is obtained by exposing embryos to methylazoxymethanol acetate through their mothers at gestational day 15. Methylazoxymethanol is an antiproliferative agent showing neuroepithelial selectivity (Cattaneo et al., 1995). As a consequence of a gestational day 15 treatment to the mother, the offsprings show a marked cellular ablation in the intermediate layers of the cortex and in the CA region of the hippocampus, in agreement with the neurogenetic gradient in rodents. Methylazoxymethanol treated rats develop normally, but in adulthood they show impairment of learning and memory. Interestingly, these neuroanatomical, behavioral and electrophysiological abnormalities have their counterpart, at the molecular level, in a consistent redistribution of protein kinase C in synaptosomes of cortex and hippocampus, the brain areas most affected by the treatment (Di Luca et al., 1995; Caputi et al., 1996). In particular the amount of protein kinase C localized in the membrane compartment of synaptosomes is significantly increased, and this increase is paralleled by an increased in vivo phosphorylation of its presynaptic substrate B-50/GAP-43 (Di Luca et al., 1993). As a consequence of the alteration in basal protein kinase C translocation, a parallel increase in calcium dependent glutamate release in methylazoxymethanol treated rats was observed (Di Luca et al., 1997). Moreover, long-term potentiation could be restored by D-serine (Ramakers et al., 1993), an agonist at the glycine site of NMDA receptors.

Methylazoxymethanol treated rats, characterized by lack of long-term potentiation in the CA1 region, show a marked decrease in the activity of Ca^{2+}/Calmodulin-dependent protein kinase II when compared to controls, although the concentration of the enzyme in PSD is not altered. This effect is confined to PSD-associated Ca^{2+}/Calmodulin-dependent protein kinase II, since the enzyme activity tested in the soluble fraction is unchanged in methylazoxymethanol treated rats (Caputi et al., 1999). In addition, the decreased activity is not due to inhibition by autophosphorylation in specific sites within the Calmodulin binding domain, since preincubation with purified phosphatases 1 and 2A failed to restore Ca^{2+}/Calmodulin-dependent protein kinase II activity in PSD of methylazoxymethanol treated rats. The phosphorylation by

Ca^{2+}/Calmodulin-dependent protein kinase II of both NR2A and NR2B subunits of NMDA receptor is also lower in methylazoxymethanol treated rats when compared to controls (51.77% ± 7.39% of controls level), as revealed in back-phosphorylation experiments (Caputi et al., 1999). The activity of Ca^{2+}/Calmodulin-dependent protein kinase II was reconstituted by incubating hippocampal slices with D-serine, a treatment previously shown to rescue long-term potentiation in methylazoxymethanol treated rats (Ramakers et al., 1993). D-serine acts as an agonist at the glycine-site of NMDA receptors and is able to prevent NMDA receptor desensitization in mouse hippocampal cultures (Vyklicky et al., 1990). The finding that it can also restore long-term potentiation in methylazoxymethanol treated rat hippocampal slices to control levels (Ramakers et al., 1993) suggests that in these animals the higher basal level of glutamate release induces NMDA receptor desensitization that can be rescued with D-serine. These data taken together point to an important role of postsynaptic Ca^{2+}/Calmodulin-dependent protein kinase II activity in modulating synaptic plasticity through phosphorylation of NMDA receptors. Its physical association with NR2 subunits, as discussed above, places it in the core of the action responsible for the long lasting changes associated with synaptic plasticity.

6. Conclusion

In conclusion, the results obtained by different authors on both the structural organization and the functional regulation of PSD suggest that PSD is a dynamic structure whose modulation plays a crucial role in synaptic plasticity. The post translational modifications of the molecular elements localized in PSD give, in fact, a molecular rationale for the sustaining of synaptic strength in short-term plasticity. However, the molecular mechanisms responsible for morphological modifications of the synapse in long-term changes of synaptic strength as well as the transduction pathway(s) transforming the initial trigger in long-term effects, remain to be explored. The presence in PSDs of proteins and enzymes involved in tyrosine kinase cascade might provide for such a link and needs further elucidation.

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