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Modification of glutamate receptors by phospholipase A2: its role in adaptive neural plasticity

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Abstract. Long-term potentiation (LTP) and long-term depression (LTD) are two electrophysiological models that have been studied extensively in recent years as they may represent basic mechanisms in many neuronal networks to store certain types of information. In several brain regions, it has been shown that these two forms of synaptic plasticity require sufficient dendritic depolarization, with the amplitude of the calcium signal being crucial for the generation of either LTP or LTD. The rise in calcium concentration mediated by the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors has been proposed to stimulate various calcium-dependent enzymatic processes that could convert the induction signal into long-lasting changes in synaptic structure; protein kinases and phosphatases have so far been considered predominantly with regard to LTP and LTD formation. According to several lines of experimental evidence, changes in synaptic function observed with LTP and LTD are thought to be the result of modifications of postsynaptic currents mediated by the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) subtype of glutamate receptors. Moreover, it has become apparent recently that activation of the calcium-dependent enzyme phospholipase A2 (PLA2) could be part of the molecular mechanisms involved in alterations of AMPA receptor properties during long-term changes in synaptic operation. In the present review, we will first describe the results that indicate a critical role of the phospholipases in regulating synaptic function. Next, sections will be devoted to the effects of PLA2 and phospholipids on the binding properties of glutamate receptors, and a revised biochemical model will be presented as an attempt to integrate the PLA2 enzyme into the mechanisms (in particular kinases and phosphatases) that participate in adaptive neural plasticity. Finally, we will review data relevant to the issue of selective changes in AMPA binding after environmental enrichment and LTP.

Key words. Glutamate; receptors; phospholipases; LTP; LTD.

Introduction

Some forms of LTP and LTD are triggered by the activation of NMDA receptors and the resulting influx of calcium into the postsynaptic structure [1–3] but appear to be expressed, at least in part, by changes in synaptic responses generated by the AMPA receptors [4–7]. Regarding synaptic potentiation, important research has revealed that LTP expression could involve the uncovering of functional AMPA receptors that, prior to LTP, are either not present in postsynaptic membranes or are silent electrophysiologically [8, 9]. Such modifications of AMPA responses have been proposed to be caused by an increase in the number of receptors, their open probability, their kinetics or their single-channel conductance [10–14]. Interestingly, biochemical evidence has shown that AMPA receptor synthesis and 3H-AMPA binding are augmented after LTP induction [12, 13, 15, 16], and LTP production in the CA1 region of the hippocampus is associated with heightened single-channel conductance of AMPA receptors [14].

Since LTP is initiated by calcium entry through the NMDA channels, we and others have postulated that the process (or processes) underlying synaptic plasticity has (or have) to be found among those that are calcium-dependent and that can produce a selective change in

AMPA receptor properties [2, 17]. Among the various calcium-dependent enzymes, protein kinases and phosphatases have so far been considered predominantly with regard to LTP and LTD formation. In particular, the levels of postsynaptic calcium influx achieved with different degrees of NMDA receptor activation during tetanic stimulation are thought to produce opposite changes in protein phosphorylation, with high levels of calcium influx activating protein kinases and generating LTP, whereas low calcium influx activates protein phosphatases and leads to LTD [18–20]. The phosphatases generally implicated in the formation of hippocampal LTD are the calcium-independent phosphatase1 and the calcium/calmodulin-dependent phosphatase calcineurin [21, 22]. This crucial role of protein kinases in synaptic plasticity has been confirmed by gene knockout experiments, and it has been shown that activation of α-calcium/calmodulin kinase II (CaM-KII) or γ-protein kinase C (PKC) is possibly important for LTP formation [23, 24], an idea consistent with the observation that LTP induction produces constitutive activation of both CaM-KII and PKC [25, 26].

Of course, there is general agreement that postsynaptic kinases are involved in the appearance of LTP, but their exact role in the maintenance of LTP remains unclear. For instance, using intracellular perfusion patch-clamp methodology (which makes it possible to introduce substances after LTP induction), Otmaklov and Lisman [27] recently demonstrated that postsynaptic inhibitors of CaM-KII block the induction but not the maintenance of LTP. In addition, studies using gene-targeting approaches have revealed that alterations in kinase activity in transgenic animals do not necessarily eliminate the formation of either LTP or LTD elicited by appropriate tetanic stimulation [18, 28, 29]. Within this context, it has been hypothesized that several other calcium-dependent enzymes, such as lipases and proteases, could be involved in producing long-term changes in synaptic efficacy [30].

Synaptic plasticity and phospholipases

A number of membrane-associated phospholipases are calcium-dependent. There is evidence linking glutamate receptor activation to phospholipases with several arguments suggesting a role for phospholipase A2 (PLA2) in LTP formation. For instance, the tetanic stimulation used to induce LTP and NMDA receptor agonists elicits the release of arachidonic acid (AA) [31–33], which indicates that NMDA receptor activation produces in situ stimulation of PLA2. In addition, NMDA receptor activation is found to evoke long-lasting enhancement of endogenous PLA2 activity [34], and several reports suggest that different compounds that inhibit PLA2 activity interfere with LTP [33, 35, 36]. In area CA1 of hippocampal slices, we demonstrated that PLA2 inhibitor interferes with LTP induction by disrupting a process (presumably the activation of endogenous PLA2 or possibly another phospholipase, as the drug is not a totally selective inhibitor) that follows the activation of ionic currents (NMDA receptor-elicited calcium currents) which serves as the initial trigger for LTP [37].

Numerous investigations have demonstrated that NMDA receptor activation is required for LTD induction, and a reduction in AMPA receptor function might be linked to maintenance of synaptic depression in area CA1 of the hippocampus [38]. It has been reported that application of arachidonate in hippocampal slices might mimic LTD formation in young animals [39], whereas preincubation of hippocampal slices with PLA2 inhibitors blocks LTD formation [36, 37, 40]. Recently, we examined the effects of several inhibitors of AA metabolism on LTD formation in hippocampal slices prepared from young rats (postnatal days 20–25). We found that the presence of baicalein, a 12-lipoxygenase inhibitor of AA metabolism, in the perfusion medium significantly decreases the magnitude of hippocampal LTD elicited by low-frequency stimulation (LFS) [40]. Indeed, there is a general agreement that 12-lipoxygenase metabolites are involved in changes of synaptic operation in invertebrate and vertebrate neurons [41], and some experimental evidence suggests that metabolites generated by 12-lipoxygenase pathways have various cellular effects, such as hyperpolarization and increased postspike hyperpolarization [42] or inhibition of CaM-KII [43, 44], which are compatible with synaptic depression in the hippocampus. It is noteworthy that inhibition of 5-lipoxygenase by AA-861 is only slightly effective in suppressing LTD in young animals, whereas indomethacin does not affect the magnitude of hippocampal LTD; on the other hand, neither cyclooxygenase nor lipoxygenase inhibitors have significant effects on the magnitude of LTP elicited by theta burst stimulation in young rats [40]. It is unclear whether the difference in drug effects on hippocampal LTD is an age- or an area-dependent phenomenon. However, as LTD expression in the cerebellum also appears to be mediated by PLA2 activation [45], it will be certainly important to determine how the formation of lipoxygenase products could be a common mechanism underlying synaptic depression in this brain structure.

The main conclusion drawn from these diverse studies is that PLA2 activation could be a common mechanism involved in both LTP and LTD expression. The direction of the changes in synaptic function elicited by PLA2 appears to be determined by distinct cellular processes associated with enzyme activation. Specifically, we are suggesting that the reduction of synaptic

transmission observed during LTD could result from the accumulation of 12-lipoxygenase metabolites of AA. In contrast, LTP formation is not related to metabolite production and could possibly be mediated by other biochemical processes associated with PLA2 activation.

Regulation of glutamate receptors by phospholipase A2 and phospholipids

Treatment of membranes with exogenous phospholipases has been reported to alter the characteristics of the binding sites for various neurotransmitters or neuromodulators such as norepinephrine, γ-aminobutyric acid and the opioid peptides. In a number of cases, it has been shown that the nonpolar or polar moieties of membrane phospholipids, as well as the lysophospholipids and fatty acids generated by phospholipase treatment, are responsible for the changes in receptor binding. In other cases, it appears more likely that modifications of the lipid microenvironment of the receptor produce alterations in conformation of the receptor, leading to changes in receptor binding [46]. Treatment of synaptic membranes with PLA2 has been shown to produce an increased affinity of the AMPA receptor for its ligand, whereas 3H-kainate, 3H-glycine and 3H-glutamate binding to the kainate and NMDA receptor, complex, respectively, is not affected [47]. Although the mechanistic details underlying the selective modulation of AMPA receptor properties by PLA2 remain to be identified, it is clear that the effect of PLA2 on 3H-AMPA binding is due to PLA2-induced disturbances in the lipid environment of the receptors, as the effect was not found to be reversed by scavengers of metabolites resulting from PLA2 stimulation.

In view of the role of the lipid microenvironment in modulating the properties of AMPA receptors, we investigated the effects of exogenous phospholipids on the characteristics of 3H-AMPA binding to rat telencephalic membranes. The various results we obtained are quite interesting. First, it was demonstrated that incorporation of phosphatidylserine (PtdSer) in neuronal membranes generated a large increase in the affinity of 3H-AMPA for its receptor. No such changes in AMPA binding were observed after incorporating phosphatidylethanolamine, phosphatidylcholine or cholesterol [48]. Recent work has also revealed that the PtdSer-induced increase in 3H-AMPA binding is different among various brain structures, being larger in the hippocampus and in the molecular layers of the cerebellum but minimal in the striatum (see fig. 1). Here again, this effect of PtdSer seems to be relatively specific, as treatment did not modify 3H-glutamate binding to the NMDA subtype of glutamate receptor [48, 49]. These findings clearly indicate that the manipulation of phospholipids by opposite means (i.e. PLA2 and PtdSer incorporation) produces the same effect on the AMPA receptor (an increase in affinity). Furthermore, they are consistent with the hypothesis that an alteration in the lipid environment of synaptic membranes could be an important mechanism for regulating AMPA receptor properties during long-lasting changes in synaptic operation.

Few studies, however, have been performed to directly evaluate the role of endogenous phospholipases in modulating the binding properties of AMPA receptors. We reported previously that potassium (KCl)-induced depolarization of rat telencephalic synaptoneurosomes produces a heightened affinity of the AMPA receptor for its agonists [50, 51]; synaptoneurosomes are pinched off nerve terminals associated with resealed postsynaptic structures, and they have been useful tools to study mechanisms of transmitter release, regulation of transmitter receptors and second-messenger pathways. We found, moreover, that the KCl-induced increase in affinity of the AMPA receptor is selective for 3H-AMPA, and is markedly reduced by the PLA2 inhibitor BPB [50, 51]. We have also addressed this important question by using melittin, a bee venom peptide, which has been shown to potently activate endogenous PLA2 [52]. The results we obtained reveal that treatment of rat brain synaptoneurosomes with melittin produces a greater affinity of the AMPA receptor for its agonist, without significantly changing 3H-MK-801 binding to NMDA receptor. Here again, the effect of melittin is significantly reduced by BPB and does not appear to be due to the generation of phospholipid metabolites; this

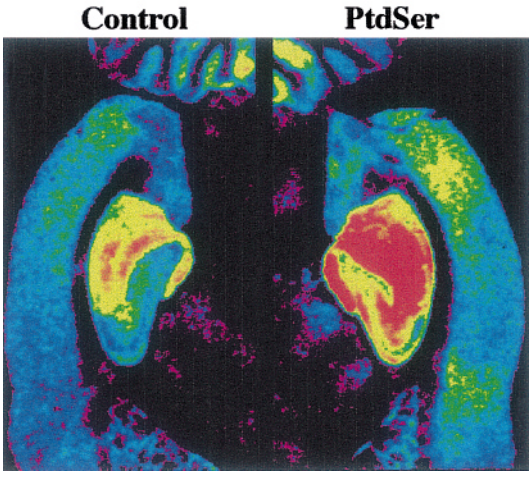

Figure 1. Effect of phosphatidylserine treatment on 3H-AMPA binding in rat brain sections. Horizontal sections were preincubated at 35 °C for 1 h in Tris-acetate buffer without (control) or with 400 nM of phosphatidylserine (PtdSer). They were then washed and processed for 3H-AMPA binding.

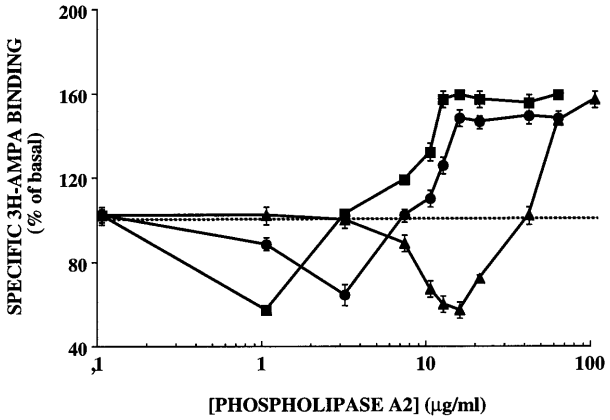

Figure 2. Effect of calcium on PLA2-induced changes in 3H-AMPA binding in rat synaptoneurosomes. Synaptoneurosomes were prepared from rat hippocampus and were preincubated in the presence of various PLA2 concentrations (0.01–100 μg/ml) and with 0.5 mM (closed triangles), 2.0 mM (closed circles) or 5.0 mM (closed squares) of calcium. After preincubation, membrane suspensions were prepared and then processed for 3H-AMPA binding (reproduced from [40]).

potential importance of PLA2 in the selective regulation of AMPA receptors was also strengthened by the observation that melittin enhances AMPA-stimulated calcium influx in cultured neurons [53].

Bidirectional control of AMPA receptors by exogenous PLA2

As PLA2 inhibitors reduce the magnitude of both LTP and LTD, we hypothesized that PLA2 activation could elicit either increased or decreased AMPA receptor function, depending on its degree of activation, thereby participating in bidirectional regulation of efficacy at glutamatergic synapses [54]. By employing rat synaptoneurosome preparations, we have demonstrated that their treatment with escalating concentrations of PLA2 produces a biphasic effect on AMPA receptor binding, with low concentrations causing a decrease and high concentrations an increase in agonist (but not antagonist) binding [40]. On the other hand, we demonstrated that the levels of calcium in synaptoneurosome suspensions determined the direction of PLA2-mediated AMPA receptor modulation. As shown in figure 2, the effects of increasing PLA2 concentrations on 3H-AMPA binding and its modulation by calcium were remarkably similar to the f function proposed by Cooper and Bear to describe changes in synaptic efficacy as a function of postsynaptic depolarization and sliding of the curve depending, on prior synaptic activity [55, 56]. It was noted that incubation with 12-lipoxygenase inhibitors preferentially reduced the PLA2-induced decrease of AMPA binding generated by low PLA2 concentrations. Moreover, treatment of hippocampal synaptoneurosomes with arachidonic acid or 12-hydroperoxyeico-satetraenoic acid (12-HPETE), the first metabolite generated from the hydrolysis of AA by 12-lipoxygenases, was found to reproduced the PLA2-induced reduction in 3H-AMPA binding [40]. Altogether, these data strongly support the notion that the same biochemical pathway, i.e., NMDA receptor activation and endogenous PLA2 stimulation, may represent a common mechanism resulting in AMPA receptor alterations during both LTP and LTD.

As mentioned previously, bidirectional regulation of synaptic function has been proposed to be mediated by a complex balance between phosphorylation and dephosphorylation reactions [18]. In particular, different observations have suggested that changes in phosphorylation of AMPA receptors produced by various kinases and phosphatases are involved in the regulation of synaptic efficacy, and earlier studies have demonstrated that phosphorylation and dephosphorylation actions of protein kinase and phosphatase lead to differential regulation in the binding properties of AMPA receptors during the developmental period [57]. Moreover, several findings support the possibility that protein kinases and phosphatases directly regulate PLA2 activity. For instance, PKC has been reported to up-regulate PLA2 activity in various preparations, and several compounds activating PKC were shown to cause phosphorylation of the serine or threonine residues of PLA2 [58].

As calcium-dependent kinases and phosphatases provide intracellular processes for the expression of several forms of synaptic plasticity, we recently tested the effects of kinase activation and phosphatase inhibition on PLA2-mediated regulation of AMPA receptor binding [40]. It was observed that 8-bromo-cyclic AMP and forskolin, which augment protein kinase A (PKA) activity, did not alter the PLA2-induced decrease or increase of 3H-AMPA binding in hippocampal synaptoneurosomes, whereas the PKC activators phorbol dibutyrate (PDBu) and phorbol 12-myristate, 13-acetate (PMA) markedly enhanced the rise in AMPA binding elicited by high PLA2 concentrations. In contrast to PKC activators, preincubation of hippocampal synaptoneurosomes in the presence of the protein phosphatase inhibitor okadaic acid did not significantly enhance the PLA2-induced increase in 3H-AMPA binding when compared with the controls, but resulted in a significant decrease in the effects of PLA2 to reduce 3H-AMPA binding. This indicates that phosphatase activation might be involved in controlling the PLA2-induced diminution of AMPA binding which is consistent with experiments showing that application of

phosphatase inhibitors caused a reduction of LTD in young animals [21, 22].

Since treatment of synaptoneurosomes with protein kinase activators augmented both PLA2 activity and the PLA2-mediated increase in AMPA binding, it is tempting to suggest that phosphorylation conditions are critical in determining the direction of PLA2-mediated changes in AMPA receptors. A putative biochemical model that accounts for the bidirectional control of synaptic function by phosphorylation processes and PLA2 activation is presented in figure 3. We first postulate that a balance between kinase and phosphatase activity determines the level of PLA2 activity. During LFS, a moderate influx of calcium, mediated by NMDA receptor activation, might cause an increase in phosphatase activity which could partially activate PLA2. Then, the lipase would downregulate AMPA receptor affinity by producing lipoxygenase products of AA, resulting in LTD. During high-frequency stimulation (HFS), a large amount of calcium would preferentially stimulate protein kinases which now fully activate PLA2. The enzyme would upregulate AMPA receptor affinity by changing the lipid environment of AMPA receptors, thereby producing LTP. Indeed, other calcium-dependent enzymes, such as calpain, could also be involved in mediating AMPA receptor changes in LTP [17]. It should be noted that, in addition to its postsynaptic actions, activation of the AA cascade could increase glutamate release through presynaptic interaction with metabotropic glutamate receptors and inhibit the function of glutamate transporters [2]. We cannot yet totally exclude the possible actions of AA or its metabolites at the presynaptic level, which could involve changes in transmitter release during both LTD and LTP.

PLA2-induced changes in 3H-AMPA binding and synaptic plasticity

Several groups have reported that epileptiform activity in the limbic system disrupts the mechanisms that induce LTP. We reasoned that such a situation might

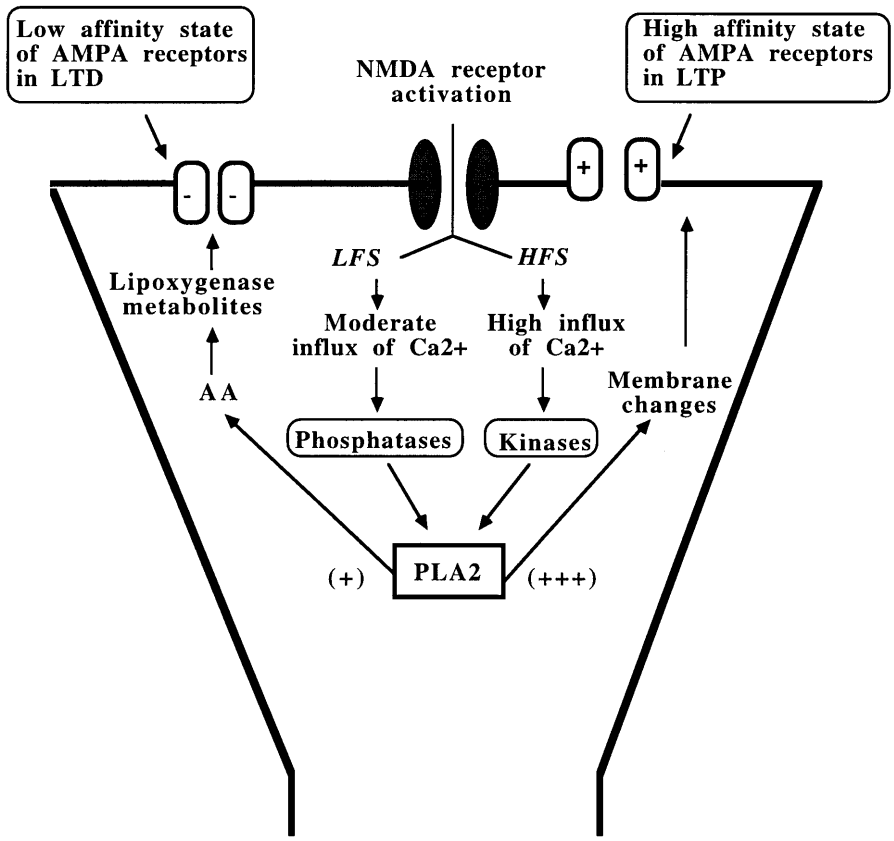

Figure 3. A biochemical model integrating phosphorylation processes and PLA2 activation for mediating synaptic plasticity (reproduced from [40]).

provide a unique opportunity to establish the roles of different calcium-dependent processes in LTP induction and especially that of calcium-dependent phospholipases. We thus examined the effects of kainate-induced epileptic seizures on the binding properties of hippocampal glutamate receptors, the modulation of AMPA receptors by PLA2 and the formation of LTP in hippocampal membranes and slices, respectively [59]. Whereas the binding of various ligands to the NMDA receptor is not modified by kainate treatment, the maximal number of binding sites for 3H-AMPA decreased notably in hippocampal membranes. More important, the increase in 3H-AMPA binding elicited by PLA2 treatment of hippocampal but not cerebellar membranes is markedly decreased following kainate injection. LTP was also substantially reduced in area CA1 after kainate treatment, and considering previous arguments supporting a role for PLA2 in LTP, it is reasonable to assume that PLA2-induced modification of the AMPA receptor is a necessary step in LTP induction. The mechanisms that underlie this decreased sensitivity of hippocampal membranes to PLA2 following kainate treatment are not known. However, we have demonstrated that systemic administration of kainate is associated with calpain activation, as the amount of spectrin breakdown products was increased severalfold in hippocampal (but not cerebellar) homogenates. This observation, combined with the fact that PLA2-induced enhancement in 3H-AMPA binding was found to be markedly reduced by calpain, raised the possibility that kainate-induced seizure activity could produce long-lasting disturbance of membrane properties as a result of massive in situ calpain activation in the hippocampus [59]; this in turn could explain why the modulation of AMPA receptors by exogenous PLA2 was not impaired in the cerebellar formation. Of course, these data also suggest that a specific order of activation of calcium-dependent processes might be important in producing changes in synaptic operation in the hippocampus.

An age-dependent regulation of AMPA receptor binding has been demonstrated in rat neocortical slices after cellular depolarization and agonist stimulation, consistent with a role for the AMPA receptors in synaptic plasticity [57]. On the other hand, developmental studies have shown that LTP in the rat hippocampus exhibits a specific developmental profile. In particular, LTP is absent in the first 8 postnatal days in area CA1 of the hippocampus and appears after postnatal days 10–11 [60, 61]. The inability of Schaffer collateral/commissural synapses of the hippocampus to display LTP in young rats could reflect altered cellular processes associated with LTP expression or the lack of mechanisms involved in LTP induction, such as calcium influx through the NMDA receptor channel. Indeed, PLA2 activity or the dissociation between PLA2 activity and receptor modulation could account for the lack of LTP in young animals. We found recently that potentiation of synaptic responses in area CA1 of the hippocampus and modulation of AMPA receptor binding in telencephalic synaptoneurosomes following KCl-induced depolarization are markedly reduced in neonatal compared with adult rats [51]. Moreover, both phenomena follow a similar developmental profile which could reflect developmental changes in the modulation of AMPA receptors by endogenous PLA2. Accordingly, telencephalic synaptoneurosomes prepared from neonatal animals exhibited a significant reduction in the ability of melittin to modulate AMPA receptor binding; and as melittin stimulated AA release as effectively in neonatal as in adult synaptoneurosomes, its reduced ability to modify AMPA receptor binding cannot be accounted for by decreased PLA2 activity in the neonatal brain [51]. Rather, it suggests that alterations in AMPA receptor properties or in the link between PLA2 activity and receptors during the developmental period are responsible for the observed effects. This interpretation is obviously consistent with the observation that PtdSer-induced changes in AMPA binding are abolished in neonatal brain sections [49]. Nevertheless, when the effect of PLA2 on 3H-AMPA binding was tested in telencephalic membranes prepared from very young animals, not only did we not detect any stimulation of 3H-AMPA binding, but a marked decrease in binding was obtained [62]. This finding is quite interesting because of previous work suggesting that LTD formation could be related to PLA2 activation [54, 63], and it is reasonable to propose that such a reduction in AMPA binding generated by the activation of endogenous PLA2 could represent an important mechanism for LTD expression in the hippocampus.

In summary, the above experiments reveal that PLA2-induced modification of AMPA receptors is not present in tissues in which LTP cannot be reproduced, such as following seizure activity or during the neonatal period. It is thus tempting to speculate that the PLA2 enzyme is an important factor that contributes to the stabilization of LTP, but this idea has yet to be tested.

Changes in 3H-AMPA binding after environmental enrichment

Studies have revealed that rats reared in an enriched environment exhibit various behavioral, electrophysiological and biochemical modifications. For instance, adult rats kept under enriched conditions show enhanced expression of neurotrophin-3 and glucocorticoid messenger RNAs (mRNAs) in the hippocampus [64, 65]. In addition, spatial learning, which is dependent on the integrity of hippocampal formation, is improved in rats previously exposed to an enriched environment

[66]. At the electrophysiological level, synaptic strength in the medial perforant pathway is increased in animals exposed to enriched conditions [67, 68], and since LTP in the medial perforant pathway of the dentate gyrus is reduced in enriched rats [67], it has been proposed that both LTP and enrichment act on the same biochemical variables responsible for altering AMPA receptor properties.

In collaboration with the laboratory of Dr Thomas C. Foster of Kentucky University, we have initiated studies aimed at evaluating the possibility that changes in the properties of glutamate receptors take place in vivo as a result of environmental enrichment. In particular, we used quantitative autoradiography to investigate alterations in the binding properties of AMPA and NMDA receptors in the rat hippocampus after environmental stimulation. Environmental enrichment was found to be associated with increased 3H-AMPA binding in the hippocampus without modification of 3H-glutamate binding to the NMDA receptor [67]. Quantitative ligand-binding autoradiography also revealed that environmental enrichment resulted in a significant and uniform decrease in the capacity of calcium and PtdSer to upregulate 3H-AMPA binding in various hippocampal regions [69]. Obviously, these data are consistent with the notion that hippocampal synaptic plasticity might be linked to modulation of AMPA receptor function by calcium-dependent mechanisms [17, 46].

It is interesting to mention that changes in AMPA receptors could also be linked to learning and memory. For instance, memory expression of step-down inhibitory avoidance was found to be accompanied by enhanced 3H-AMPA binding to its receptors in the rat hippocampus up to 2 h after training [70], and AMPA binding increased in the rat hippocampus several hours after eye-blink conditioning [71]. The question of an exact relationship between the magnitude of LTP and the performance in hippocampal-dependent learning tasks is still highly debated and controversial [72], because it has been proven difficult to assign the memory deficit for specific learning tasks solely to the lack of LTP in the hippocampus. However, Baudry and his group have observed that LTP can induce increased AMPA binding in the hippocampus in a manner analogous to learning, which certainly endorses the idea that LTP is crucial (if not the basis) for memory [16]. On the other hand, a selective reduction in 3H-AMPA binding was noted in the rabbit cerebellum following classical conditioning of the eyelid-nictitating membrane response [73]. This observation is indeed consistent with the idea that LTD at parallel fiber-Purkinje cell synapses, mediated by a reduction in AMPA receptor properties [74], is a form of synaptic plasticity that supports this type of learning. Work is required to evaluate the potential involvement of PLA2 in regulating changes of AMPA receptor properties after learning and LTP in vivo.

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