Functional Regulation of Glutamate Receptor GluR1: Long Term Potentiation and Depression of GluR1 by Post Translational Modification

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Abstract.- Long-term potentiation (LTP) and – depression (LTD) are the major mechanisms in memory and learning. These are regulated by post-translational modification (PTM) such as phosphorylation and glycosylation. These different PTMs are dynamic and result in temporary conformational changes that regulate many functions of the protein. Defining these PTMs in vivo is very difficult. For this purpose computational tools are very useful and show a relatively high accuracy for determination of PTMs. In this work, functional importance of PTMs, phosphorylation and O-GlcNAc modification of the glutamate receptor GluR1 is investigated. This in silico study showed that during LTP GluR1 is phosphorylated and in LTD GluR1 is O-GlcNAc modified. This interplay between these two modifications contribute to the process of memory and learning.

Keywords: Mammalian glutamate receptor GluR1, phosphorylation, O-linked Glycosylation, long-term potentiation and depression, memory and learning.

INTRODUCTION

The process of long term potentiation (LTP), communication between two neurons stimulated simultaneously in synaptic plasticity, has been comprehensively studied and its mechanism is well defined (Cooke and Bliss, 2006). LTP can be divided into two phases: An early LTP (E-LTP), which is an inducing synaptic strength, and late LTP (L-LTP) that is maintenance of excitatory synaptic strength for a long time. This perspective makes LTP parallel to memory (Lynch, 2004). E-LTP is associated with short-term memory, whereas long-term memory is linked to L-LTP (Lynch, 2004). Long term depression (LTD), which is a prolonged weakening of a neuronal synapse, is reverse of LTP. The internal switching between LTP and LTD are the main mechanisms that control synaptic plasticity, and these events are regulated by post translational modifications (PTMs) of synaptic proteins.

PTMs of proteins temporarily modify their function by reversibly transforming the structure...
calcium/calmodulin-dependent protein kinase II (CaMK-II) and protein kinase C (PKC) (Malenka, 1994; Malenka and Nicoll, 1999) triggering several signaling pathways such as RAS and RAP signal pathways (Zhu et al., 2002). These various events eventually lead to transcription in the nucleus of Cre-containing genes in L-LTP (Cooke and Bliss, 2006). The newly synthesized proteins, includes AMPAR subunits, some transcriptional factors, and structural proteins that strengthen the synapses already established and increase the number of spines on dendrites to form new synapse (Fukazawa et al., 2003; Matus, 2000).

The AMPARs include four subunits GluR1-4 that form a heterotetrameric structure (Collingridge et al., 2004). These are expressed abundantly throughout the brain, particularly in cerebral cortex, basal ganglia, thalamus and hypothalamus, hippocampus, cerebellum and spinal cord (Dure and Young, 1995; Blackstone et al., 1992). The trafficking and cellular distribution of AMPARs regulate the neuronal signal transmission (Esteban, 2003), which is controlled by PTMs specifically of the receptor subunit GluR1. This mechanism regulation is an important factor in the induction of LTP (Malinow, 2003; Barry and Ziff, 2002). Phosphorylation of GluR1 in its C-terminal domain is known to occur. PKC and CaMK-II phosphorylate Ser 831 (Mammen et al., 1997; Roche et al., 1996), and protein kinase A (PKA) phosphorylates Ser 845 in GluR1 (Roche et al., 1996). Phosphorylation of Ser 831 and 845 play an important regulatory role in the trafficking of AMPARs in LTP and LTD.

O-Glycosylation is another vital PTM that is known to play a crucial role in synaptic plasticity. O-linked N-acetylglucosamine (O-GlcNAc) modification occurs on nuclear and cytoplasmic proteins (Comer and Hart, 2000). O-GlcNAc transferase (OGT) transfers O-GlcNAc to Ser or Thr residues in proteins, and is abundant in synaptosomes, where it is known to regulate synaptic proteins (Cole and Hart, 2001). It is generally believed that O-GlcNAc modification represses transcription, but in some instances O-GlcNAc has been shown to be an activator of transcription (Gewinner et al., 2004; Kreppel et al., 1997; Lubas et al., 1997). The interplay between O-GlcNAc modification and phosphorylation are found to be reciprocal on the same or neighboring Ser/Thr residues in the protein, also known as Yin Yang sites (Kelly et al., 1993). This inverse relationship has been shown to regulate the process of transcription and translation (Chou et al., 1995; Datta et al., 1989), and is an important factor in diabetes (Baron et al., 1995) and many neurodegenerative diseases (Arnold et al., 1996).

In this *in silico* study, the interplay between O-GlcNAc modification and phosphorylation of GluR1 is studied. The results show that during LTP GluR1 is phosphorylated on Ser 831 and 845, whereas during LTD GluR1 is O-GlcNAc modified on same amino acids. This *in silico* work suggests that the interplay between these modifications provide a switch between LTP and LTD, and is an important and vital mechanism in memory and learning.

**MATERIALS AND METHODS**

The sequence of the glutamate receptor subunit GluR1 was retrieved from Swiss-prot database with entry name GRIA1_MOUSE and primary accession no. P23818 (Boeckmann et al., 2003). The sequence was searched against NCBI database by using BLAST for similar sequences in all organisms (Altschul et al., 1997). All sequences with zero expect value were selected to search the conserved Ser/Thr or Tyr residues in GluR1. The selected sequences were Q38PU8.1 from *Macaca fascicularis*, NP_001001774.1 from *Gallus gallus*, AAI53333.1 from *Xenopus (Silurana) tropicalis*, NP_113796.1 from *Rattus norvegicus*, ACJ64117.1 from *Columba livia* and AAA58613.1 from *Homo sapiens*. ClustalW was used for multiple alignments of selected sequences with the sequence of *Mus musculus* (Thompson et al., 1994).

For predicting potential for phosphorylation and O–glycosylation sites Netphos 2.0 (Blom et al., 1999) and YinOYang 1.2 (http://www.cbs.dtu.dk/services/YinOYang) (unpublished) servers were utilized. Both tools are based on artificial neural network approach. Artificial neural network are used to predict a function or elucidate patterns from complex data set, which may not be possible empirically. It is also helpful in defining
the relationship between inputs and outputs.

The *Mus musculus* sequence was submitted to the Netphos 2.0 server, which calculates the phosphorylation potential for each Ser, Thr and Tyr residue. If the predicted score was higher than 0.5, the threshold value, the site was considered as potential phosphorylation site and vice versa. The YinOYang 1.2 server predicts O-GlcNAc modification and Yin Yang sites (sites where an interplay between phosphorylation and O-GlcNAc modification occurs). The threshold value varies and depends upon surface accessibility of the residue.

Table I. *In silico* predicted phosphorylation –, O-GlcNAc – and Yin Yang sites in *Mus musculus* GluR1.

<table>
<thead>
<tr>
<th></th>
<th>Ser</th>
<th>Thr</th>
<th>Tyr</th>
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<tr>
<td>O-GlcNAc sites</td>
<td>18, 255, 627, 845, 863.</td>
<td>271, 375, 453, 560, 770, 838.</td>
<td></td>
</tr>
<tr>
<td>Yin Yang sites</td>
<td>18, 255, 627, 845, 863.</td>
<td>560.</td>
<td></td>
</tr>
<tr>
<td>False Negative Yin Yang sites</td>
<td>831.</td>
<td>-</td>
<td>-</td>
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RESULTS

The *in silico* predicted phosphorylation sites are given in Table I. The result showed a total of 58 phosphorylation sites. Amongst these were 32 phosphorylated Ser, 13 phosphorylated Thr and 13 phosphorylated Tyr residues. The results are illustrated in Figure 1. These results showed a high phosphorylation potential in GluR1.

The prediction of O-GlcNAc modification sites in *Mus musculus* are given in Table I and Figure 2. A total of 11 O-GlcNAc modification sites were predicted. Out of these sites 6 sites were predicted as Yin Yang sites, which is illustrated in Fig. 3, and tabulated in Table I. The Yin Yang sites Ser 18 and Ser 627 are conserved in all selected species of mammals, aves and amphibians, whereas Ser 863 is conserved in mammals and aves and Ser 255, Ser 845 and Thr 560 are conserved in mammals (Fig. 4). Phosphorylation of Ser 831 is an experimentally verified site (Crombag *et al.*, 2008). This site shows a high potential for O-GlcNAc modification very close to the threshold value (Fig.3), and is fully conserved in mammals, aves and amphibians. These results indicate that this site is a potential false-negative Yin Yang site, and OGT and kinases may both have equal accessibility to modify Ser 831.

DISCUSSION

Memory, retention of knowledge acquired through learning, is considered to be regulated by changes in synaptic plasticity (Kandel, 1997; Alkon and Nelson, 1990; Eccles, 1964; Hebb, 1949). Different PTMs such as phosphorylation, acetylation, palmitoylation, glycosylation etc. play a critical role in synaptic plasticity. LTP and LTD are controlled by PTMs, which are the main mechanisms in learning and memory (Braunewell and Hanahan-Vaughan, 2001; Miller and Mayford, 1999; Bliss and Gardner-Medwin, 1973; Bliss and Lømo, 1973).

The receptors, N-methyl-D-aspartate receptors (NMDARs) and AMPARs, are involved in fast synaptic transmission throughout mammalian brain (Collingridge and Lester, 1989; Bleakman and Lodge, 1998). Trafficking of AMPARs to and from synapses regulates LTP (Malinow, 2003). This function is controlled by PTMs. In particular phosphorylation of the AMPAR subunit GluR1 is known to control the trafficking of the receptor from extra-synaptic sites to the synapses (Passafaro *et al.*, 2001; Oh *et al.*, 2006). Mutations in GluRI
phosphorylation sites (Ser 831 and 845) show and consequently affect spatial learning in mice impairment in the two mechanisms, LTP and LTD.

Fig. 1. Graphic representation of the potential for phosphorylation in Mus musculus GluR1. The green, red and blue lines show the potential for phosphorylation in GluR1 and the grey line represents the threshold value.

Fig. 2. Graphic representation of the potential for O-GlcNAc modification in Mus musculus GluR1. The green lines show the potential for O-GlcNAc modification in GluR1 and the blue line represents the threshold value.

Fig. 3. Graphic representation of the potential for O-GlcNAc modification and Yin Yang sites in Mus musculus.
GluR1. The green lines show the potential for O-GlcNAc modification in GluR1, the red asterisk show the positively predicted Yin Yang sites and the black asterisk show the false-negative Yin Yang site in GluR1. The blue line represents the threshold value.

Fig. 4. Multiple alignments of Mus musculus GluR1 with three mammalian, 2 avian and 1 amphibian sequences. The consensus sequence is marked by an asterisk, conserved substitution by a double dot and semiconserved
substitution by a single dot. The different sequences are ordered as in aligned results from CLUSTALW. The positively predicted Yin Yang sites are highlighted in yellow, and the negatively predicted Yin Yang sites are highlighted in green.

Fig. 5. The interplay between O-GlcNAc modification and phosphorylation in GluR1 in mammals. A decreased phosphorylation of Ser 845 and increased in glycosylation is observed during LTD. Furthermore, an increase phosphorylation of Ser 831 during LTP is observed. The potentiated synapses show decreased phosphorylation of Ser 831 and increased glycosylation during LTD and in depressed synapses the induction of LTP increases phosphorylation of Ser 845. A molecular switch between LTD and LTP is regulated by the interplay between phosphorylation and O-GlcNAc modification on Ser 831 and 845 in GluR1.

Fig. 5. The interplay between O-GlcNAc modification and phosphorylation in GluR1 in mammals. A decreased phosphorylation of Ser 845 and increased in glycosylation is observed during LTD. Furthermore, an increase phosphorylation of Ser 831 during LTP is observed. The potentiated synapses show decreased phosphorylation of Ser 831 and increased glycosylation during LTD and in depressed synapses the induction of LTP increases phosphorylation of Ser 845. A molecular switch between LTD and LTP is regulated by the interplay between phosphorylation and O-GlcNAc modification on Ser 831 and 845 in GluR1.

Phosphorylation of Ser 818, 831 and 845 are experimentally known sites, which are important in the functional regulation of GluR1 (Jiang et al., 2007; Roche et al., 1996; Mammen et al., 1997). Phosphorylation of Ser 818 is important in the insertion of AMPAR into postsynaptic membrane which is essential in LTP (Boehm et al., 2006). This site is predicted as a potential in silico phosphorylation site, but showed no potential for O-GlcNAc modification (Table I, Fig. 3). The other experimental known site Ser 831 is phosphorylated by PKC and CaMK-II in vitro (Roche et al., 1996; Mammen et al., 1997). This site is a false-negative Yin Yang site, as this site is a fully conserved site (Fig. 4) and showed a potential near the threshold for O-GlcNAc modification (Table I, Fig. 3). Serine 845 is phosphorylated by PKA in vitro (Roche et al., 1996; Lee et al., 2000). This site is a predicted in silico Yin Yang site (Table I, Fig. 3). Phosphorylation of both sites (Ser 831 and 845) is important in receptor trafficking and consequently LTD and LTP.

O-GlcNAc is highly abundant in the hippocampus, which is a region that is implicated in learning and memory (Liu et al., 2004). Several proteins are reported as O-GlcNAc modified proteins (Haltiwanger et al., 1998; Dong et al., 1996; Querfurth and Selkoe, 1994). Furthermore the interplay between O-GlcNAc modification and phosphorylation in synapsin I and II contribute to hippocampal synaptic plasticity (Tallent et al., 2009).

Naïve synapses show decreased phosphorylation of Ser 845 during LTD and increased phosphorylation of Ser 831 during LTP. The potentiated synapses on the other hand, show decreased phosphorylation of Ser 831 during LTD, and in depressed synapses the induction of LTP increases phosphorylation of Ser 845 (Jiang et al., 2006-07). During this process, dephosphorylation is introduced by protein phosphatase (PP) 1/2A (Lee et al., 2000). As these sites are Yin Yang sites, a possible interplay between phosphorylation and O-GlcNAc modification may occur on these sites. This suggests that during LTP GluR1 is phosphorylated on Ser 831 and 845, whereas during LTD both sites are O-GlcNAc modified (Fig. 5). Elevated levels of Ca²⁺ increase induction of LTP by activating CaMK-II and PKC, and decreases levels of O-GlcNAc (Griffith and Schmitz, 1999). Furthermore an interplay between OGT and PKC, PKA and OGT, CaMK-II and OGT is known (Tallent et al., 2009; Griffith and Schmitz, 1999). This shows that the role of phosphorylation and O-GlcNAc modification in hippocampal neurons regulate the functional switch between LTP and LTD.

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