

## PERSPECTIVES IN PHYSIOLOGY

### Phosphorylation targets the functional gating of a glutamate channel

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Serine/threonine and tyrosine phosphoprotein kinases and phosphatases are known to directly phosphorylate and dephosphorylate members of the three major classes of ionotropic glutamate receptors (AMPA, NMDA and kainate channels) (Moss & Smart, 1996). These enzymes also play a central role in various forms of synaptic plasticity such as long-term potentiation and depression. Therefore, it is tempting to suggest that phosphorylation might cause a long-term change in glutamate receptor function. This might be achieved through the insertion of new receptors into the subsynaptic membrane or through an alteration in the behaviour of pre-existing channels. In this issue of *The Journal of Physiology*, the paper by Traynelis & Wahl (1997) provides evidence that phosphorylation can modulate the intrinsic coupling between the binding of glutamate and the gating of the channel.

GluR6 is a member of the kainate subclass of glutamate receptors and this subunit forms functional homomeric channels when expressed in HEK 293 cells. The GluR6 protein demonstrates a relatively low level of basal phosphorylation but application of the adenylate cyclase activator, forskolin, or the coexpression of the catalytic subunit of PKA ( $\alpha$ -PKA) directly enhances receptor phosphorylation (Raymond *et al.* 1993). Furthermore, site directed mutagenesis of a serine residue to an alanine (S684A) greatly reduces phosphorylation of the GluR6 subunit. Functionally, the introduction of  $\alpha$ -PKA via whole cell patch pipettes enhances agonist-induced currents (Raymond *et al.* 1993; Wang *et al.* 1993) by increasing the apparent maximal response (Wang *et al.* 1993). In contrast, the apparent affinity of the receptor for agonist, the rise time of the responses and receptor desensitization were unchanged (Raymond *et al.* 1993; Wang *et al.* 1993). This suggests that phosphorylation may have increased the number of active channels expressed in the cells perhaps by recruiting new channels to the membrane surface or by converting pre-existing channels from a low to a high state of activity. However, phosphorylation at S684 may not be directly responsible as this site is likely to be located on an extracellular region of the protein.

The probability of channel opening is often measured under steady-state conditions (i.e. single channel recordings during a prolonged application of low concentrations of agonist). The probability of opening of the channel is calculated by measuring the total time the channels remain open divided by the duration of the recording and the estimated number of channels in the patch. In practice, it is extremely difficult to determine accurately how many channels are physically present in a patch. Furthermore, desensitization may cause receptors to enter long-lasting closed states which can be an additional confounding factor. Instead of using direct single channel current measurements to study the mechanism responsible for the PKA-dependent enhancement of GluR6 channels, Traynelis & Wahl (1997) have analysed glutamate responses using 'non-stationary current variance analysis' (Sigworth, 1980). In this application the technique provides several important advantages. Firstly, responses to rapid, brief applications of a saturating concentration of glutamate can be examined at a time when channels are largely non-desensitized. This condition more accurately reflects the peak of an excitatory postsynaptic current (EPSC). Secondly, the probability of channel opening calculated using this technique is independent of the number of channels in the patch. In addition, the weighted single channel conductance takes into account both the frequency and amplitude of all conductance states of the channel (subconductance as well as the main conductance state).

Traynelis & Wahl (1997) demonstrated that the probability of the channels opening at the peak of the response was relatively high (0.65). However, the probability increased further (0.85) when patches were exposed to  $\alpha$ -PKA. An enhanced probability of opening was also observed when patches were taken from cells coexpressing  $\alpha$ -PKA (0.94). In contrast, no change in the size of the unitary current was observed nor was there any change in the kinetics of the response or the rate of desensitization. In addition, applications of calcineurin (PP2B), a phosphatase known to be colocalized with PKA and A kinase anchoring proteins, reduced the probability of opening from control values. The most parsimonious explanation for these results is that phosphorylation increased the rate at which the receptor moved from the bound to the open state. Therefore, an increase in the maximal response to L-glutamate can be accounted for on the basis of change in the probability of opening of the channels without having to propose an increase in the number of receptors. This is important because postsynaptic receptors are likely to be exposed to

near maximal concentrations of L-glutamate during synaptic transmission. But do kainate receptors actually contribute to synaptic transmission? In fact, recent work has shown that kainate receptors can contribute to the EPSC at mossy fibre synapses in the CA3 region of the hippocampus (Castillo *et al.* 1997; Vignes & Collingridge, 1997). Nevertheless, most synapses utilize AMPA and not kainate receptors. Might the probability of opening of AMPA receptors be similarly regulated by phosphorylation? Certainly evidence suggests that kinases such as PKC, CamII and PKA can phosphorylate and enhance the function of these receptors (Wang *et al.* 1994; Roche *et al.* 1996). Furthermore, at some apparently 'silent synapses' AMPA receptors are apparently lacking or exist in an inactive form until stimulated by the induction of long-term potentiation. One proposed mechanism for this change is a prolonged increase in the phosphorylation of AMPA receptors by CamKII (Barria *et al.* 1997). Inhibition of endogenous phosphatase activity or enhancing kinase activity could potentially induce a long-term change in the probability of the gating of AMPA receptors, perhaps restoring a 'silent synapse' to full function.

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