

LETTER TO THE EDITOR

Analysis of the Presynaptic Signaling Mechanisms Underlying the Inhibition of LTP in Rat Dentate Gyrus by the Tyrosine Kinase Inhibitor, Genistein

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To the Editor:

In our opinion, the article entitled “Analysis of the Presynaptic Signaling Mechanisms Underlying the inhibition of LTP in Rat Dentate Gyrus by the Tyrosine Kinase Inhibitor, Genistein,” by Casey et al. (2002), presents weakly supported results that do not allow the interpretation suggested by the authors. In this report, Casey and colleagues investigated a putative presynaptic role for tyrosine kinases on long-term potentiation (LTP) in perforant path-granule cell synapses. An increase in KCl-stimulated Ca^{2+} influx and concomitant glutamate release was observed in synaptosomes isolated from dentate gyrus that had sustained LTP after tetanic stimulation, as compared with untetanzed tissue. This was accompanied by an increase in tyrosine phosphorylation of the $\alpha 1$ -subunit of voltage-gated calcium channels (VGCC) and of the phosphorylation of extracellular signal-regulated kinase (ERK) in synaptosomes from tetanized dentate gyrus.

Increased protein synthesis and phosphorylation of the transcription factor cAMP response element binding protein (CREB) were also observed in the input cell bodies at the entorhinal cortex. To relate these tetanus-induced events to tyrosine kinase activity, the broad-range inhibitor, genistein, was used. Intracerebroventricular injection of the drug, before tetanic stimulation, inhibited LTP *in vivo* as well as LTP-associated increases in glutamate release, Ca^{2+} influx, phosphorylation of the presynaptic proteins mentioned above, and protein synthesis. Both genistein and PD098,059, an inhibitor of the kinase that activates ERK (MEK), directly applied to untetanzed synaptosomes, also inhibited the release of KCl-stimulated Ca^{2+} influx and glutamate. Based on these results, the authors concluded that tyrosine phosphorylation of certain presynaptic proteins modulates glutamate release, thereby contributing to the expression of LTP.

We believe that the authors cannot draw this conclusion based on the data presented. The conclusions of this study that introduce novelty, *i.e.*, a role for tyrosine kinases in LTP through a presynaptic action, are based solely on the use of genistein. No attempt to prove the specificity of this drug was made, by using

inactive analogues or alternative tyrosine kinase inhibitors. This is particularly important, as several nonspecific effects have been reported for genistein. This compound inhibits voltage-sensitive Na^+ channels (Paillart et al., 1997), cardiac L-type Ca^{2+} channels (Chiang et al., 1996), myocyte delayed-rectifier K^+ currents (Washizuka et al., 1998), GABA_A and glycine receptors (Dunne et al., 1998; Huang et al., 1999; Huang and Dillon, 2000) and activates cystic fibrosis transmembrane conductance regulator Cl^- channels (French et al., 1997; Weinreich et al., 1997) in a tyrosine kinase-independent manner. Furthermore, we recently showed that genistein inhibits KCl-induced Ca^{2+} influx and glutamate release from hippocampal synaptosomes in a nonspecific manner, since two other tyrosine kinase inhibitors and two tyrosine phosphatase inhibitors had no significant effect on the same events (Pereira et al., 2003). A nonspecific inhibition of Ca^{2+} influx by genistein could account for the inhibition of glutamate release and the decrease in tetanus-stimulated protein phosphorylation and LTP, reported by Casey and colleagues. Therefore, it is not possible to infer a role for tyrosine kinases in modulating glutamate release and LTP, using genistein, as the authors did.

Moreover, we recently reported that PD098,059 inhibits glutamate release from hippocampal synaptosomes as a result of nonspecific inhibition of Ca^{2+} influx, which is not affected by U0126, another MEK inhibitor (Pereira et al., 2002). Although we used higher concentrations (10–60 μM), which caused nonetheless submaximal ERK inhibition, it is not wise to conclude that PD098,059 is acting through specific MEK inhibition in this study. In fact, this report did not verify whether PD098,059 actually inhibited ERK activity at such a low concentration (2 μM). Therefore, the conclusion drawn by Casey and colleagues that ERK inhibition results in reduced KCl-stimulated glutamate release from dentate gyrus synaptosomes based solely on the use of PD098,059, is again questionable.

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In conclusion, the report by Casey and colleagues is based on the use of two pharmacological inhibitors that have nonspecific effects and no attempt was made to verify their specificity. This led the authors to ambitious conclusions on the involvement of tyrosine kinases on LTP in perforant path-granule cell synapses, that cannot be inferred from the data presented in this article.

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