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Endothelial nitric oxide synthase and LTP

Long-term potentiation (LTP) is often considered to be a cellular correlate of learning. During LTP induction in the CA1 region of the hippocampus, nitric oxide (NO) synthesized in the dendrites of pyramidal cells may carry retrograde signals from the postsynapic to the presynaptic terminals[1,2]. We show that LTP is defective in hippocampal slices from mice lacking functional endothelial nitric oxide synthase (eNOS) and from wild-type mice treated with a NOS inhibitor. The endothelial isoform of NOS seems to be required for the maintenance of LTP in the hippocampus.

Extracellular application of NOS inhibitors blocks LTP in the hippocampal CA1 area[3–5], although there are reports that LTP generated by strong stimuli is not sensitive to these drugs[6–8]. Furthermore, in cultured hippocampal neurons, LTP is blocked by extracellular post- and presynaptic application of oxymyoglobin (which binds free NO), or by post-, but not presynaptic injection of a NOS inhibitor[9]. Exogenous NO paired with a weak, sub-threshold tetanic stimulus also induces LTP[9].

Mice lacking a functional copy of the neuron-specific NOS isoform (nNOS) exhibit normal LTP, but LTP in nNOS knockouts is blocked by NOS inhibitors[8]. This may be explained by the finding that eNOS, initially believed to be present only in endothelial cells, is the main isoform in CA1 pyramidal cells[10]. Consequently, eNOS, not nNOS, may be responsible for synthesizing NO postsynaptically during LTP. Indeed, injecting hippocampal slices with an adenovirus vector containing a truncated, and hence not functional, eNOS gene (a putative dominant negative) blocks LTP at synapses in the CA1 stratum radiatum[11].

We inactivated the eNOS gene by replacing exons 24 and 25 with the neomycin-resistance gene in the embryonic stem cell line E14-1. Functional inactivation of eNOS was demonstrated by the lack of endothelial NO formation in eNOS$^{-/-}$ mice, derived from two independently generated mutant clones. The hippocampi of these eNOS-deficient animals had no obvious anatomical defects and apparently normal excitatory synaptic transmission in the CA1 region. Baseline test excitatory postsynaptic potential (EPSP) amplitude (30–40% of the maximum EPSP amplitude), was not significantly different for wild-type and eNOS$^{-/-}$ mice (0.95±0.05 mV for control slices, 1.08±0.07 mV for eNOS$^{-/-}$ slices, mean±s.e.m.).

We chose a relatively weak LTP induction method (so as not to induce NO-independent LTP) that was highly sensitive to the NOS inhibitor *N*-nitro-L-arginine (NOARG). The method induced moderate potentiation in wild-type slices that lasted at least 90 min (148.4±12.8% 90 min after tetanus, $n=11$). In the presence of NOARG, EPSPs exhibited short-term potentiation (STP) but not LTP, decaying gradually to baseline in less than 90 min (94.9±4.2% 90 min after tetanus, $n=7$, Fig. 1a, b). After 90 min, the NOARG-treated group was significantly different from controls ($P<0.01$) but not significantly different from baseline. Using the same induction method, the eNOS$^{-/-}$ mice exhibited STP but no LTP (101.6±6.5% 90 min after tetanus, $n=10$, Fig. 1a, b). After 90 min, the potentiation level in the eNOS-deficient group was significantly different from wild-type slices ($P<0.01$), and not significantly different from baseline.

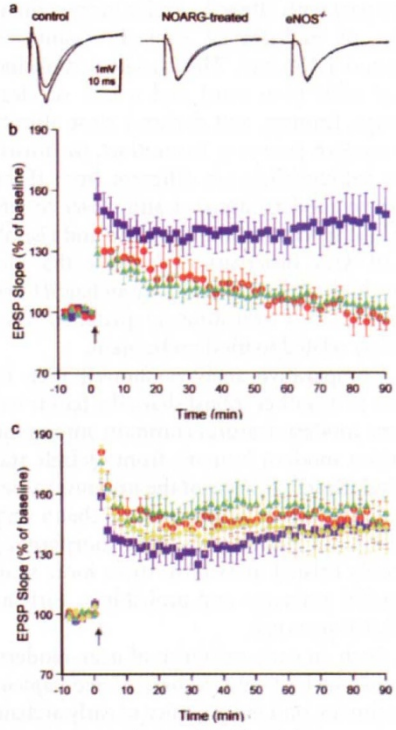

Figure 1 a, **Superimposed field EPSPs, one recorded immediately before tetanus and one 90 min after tetanus, for wild-type slices, wild-type slices treated with 200 μM NOARG, and eNOS$^{-/-}$ slices.** b, **Time course of mean normalized EPSP slopes in wild-type slices (blue squares), wild-type slices treated with 200 μM NOARG (red circles), and eNOS$^{-/-}$ slices (green triangles) subjected to weak tetanic stimulation (3 trains of 10 pulses each, 100 Hz, 20 s inter-train interval, 40 μs pulses).** c, **Time course of mean normalized EPSP slopes in wild-type slices (blue squares), wild-type slices treated with 200 μM NOARG (red circles), eNOS$^{-/-}$ slices (green triangles), and eNOS$^{-/-}$ slices treated with 200 μM NOARG (yellow diamonds) subjected to strong tetanic stimulation (80 μs pulses). Tetanic stimulation is indicated by arrows. Vertical bars show s.e.m.**

Next, we examined LTP induced by a stronger tetanic stimulation. We found that doubling the pulse duration during tetanus only (from 40 to 80 μs) induced a level of LTP that was not significantly different in wild-type slices treated with NOARG compared to wild-type slices not treated with the drug (143±11.8% 90 min after tetanus for NOARG-treated slices, $n=12$; 143±8.2% for controls not treated with NOARG, $n=8$, Fig. 1c). Using this tetanic stimulation, we observed robust LTP in eNOS$^{-/-}$ slices (150±8.6%, $n=9$) that was not significantly different at 90 min from LTP in wild-type slices or in eNOS$^{-/-}$ slices treated with NOARG (142±13.3%, $n=10$, Fig. 1c).

Our data are consistent with the hypothesis that NO synthesized by eNOS in postsynaptic CA1 pyramidal cells is a retrograde messenger required for the long-term maintenance, but not induction, of potentiation induced by weak stimuli. LTP induced by stronger stimuli does not seem to require NO, possibly because strong stimuli produce retrograde messengers in addition to NO. As LTP induced by strong stimuli in eNOS$^{-/-}$ slices is not attenuated by NOARG, it seems unlikely that the LTP we observe is due to a compensatory activity of nNOS. The resemblance between our results for eNOS$^{-/-}$ slices and for NOARG-treated wild-type slices suggests that eNOS is the main isoform participating in this process, and that any contribution from another isoform would be minimal.

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