

## Capacitance Calcium Entry Induces Hippocampal Long Term Potentiation in the Absence of Presenilin-1\*

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**Presenilins, whose mutant forms are the most common cause of early onset familial Alzheimer's disease, are involved in two very distinct processes: (i) proteolytic activity as  $\gamma$ -secretase acting on amyloid precursor protein to produce amyloid peptides and (ii) storage of  $\text{Ca}^{2+}$  in the endoplasmic reticulum (ER). In particular, absence of presenilin-1 (PS1) was claimed to potentiate capacitance calcium entry (CCE), i.e. the mechanism of replenishment of ER  $\text{Ca}^{2+}$  stores. However, until now, evidence in favor of the latter role has been obtained only in isolated or cultured cells and not on neurons *in situ*. Here, we studied the strength of the synapses between Schaffer's collaterals and CA1 neurons in hippocampal slices when they were submitted first to  $\text{Ca}^{2+}$ -free medium containing thapsigargin and subsequently to normal artificial cerebrospinal fluid, a procedure known to trigger CCE. We demonstrate that  $\text{Ca}^{2+}$  influx via the CCE mechanism is sufficient to trigger robust long term potentiation of the synapses in hippocampal slices from transgenic mice with a postnatal, neuron-specific ablation of PS1, but remarkably not from wild-type mice. Our data establish for the first time in neurons confined in normal neuronal networks that PS1 acts on the refilling mechanism of ER  $\text{Ca}^{2+}$  stores.**

Mutations in presenilin-1 (PS1)<sup>1</sup> and presenilin-2 (PS2) are the most common cause of early onset cases of familial Alzheimer's disease (1–3). Presenilins are serpentine proteins containing 6–8 putative transmembrane domains (4, 5) that are localized predominantly in the membranes of the endoplasmic

reticulum (ER) (6–8). Functional evidence demonstrates that presenilins are involved in two very distinct types of processes. Best documented is the proteolytic activity first discovered as acting on the amyloid precursor protein (APP) in producing the amyloid peptides (9), the major constituents of amyloid plaques in the brain of Alzheimer's disease patients. Presenilins are also involved in calcium signaling in neurons and other cells (10–13).

Cells store calcium ions in the ER (for a review, see Ref. 14) by the action of  $\text{Ca}^{2+}$ -transporting ATPases (i.e. SERCA), that are irreversibly blocked by thapsigargin (TG) (15). Declining  $\text{Ca}^{2+}$  stores in the ER produce a signal, the molecular nature of which is still a matter of debate (16), to open store-operated  $\text{Ca}^{2+}$  channels (SOC) in the plasma membrane (17). These activated channels serve to replenish the ER  $\text{Ca}^{2+}$  stores by the mechanism known as "capacitance calcium entry" (CCE) (18).

From experiments carried out on isolated or cultured cells, it has been proposed that presenilins exert different effects on the  $\text{Ca}^{2+}$  store system. First, deficiency of PS1 potentiates CCE (10), whereas mutant PS1 causes a deficit in CCE (10–12). Secondly, the ER calcium content, assessed by the rise in cytosolic [ $\text{Ca}^{2+}$ ] induced by thapsigargin in  $\text{Ca}^{2+}$ -free medium, is significantly decreased in cells lacking PS1 (12) and increased by a mutant PS1 (11). Thirdly, inositol triphosphate-mediated release of  $\text{Ca}^{2+}$  from the ER is decreased in PS1-deficient cells (12).

However, until now, the modulation of the  $\text{Ca}^{2+}$  store system by PS1 has not been studied in neurons *in situ*, a more physiological preparation. Here, we aimed to study the proposed role of PS1 in calcium signaling in neurons confined within normal neuronal networks, not by direct  $\text{Ca}^{2+}$  measurements but by an indirect mean, more appropriate to studies in slices from adult animals.

The synaptic efficiency between Schaffer's collaterals and CA1 neurons in hippocampal slices was assessed in circumstances that trigger entry of  $\text{Ca}^{2+}$  by the CCE mechanism. This provided a suitable system to define the effect of increased CCE in neurons that are deficient in PS1 as opposed to wild-type neurons. Based on two premises, i.e. (i) long term potentiation (LTP) induced by electrical tetanus in CA1 neurons is dependent on  $\text{Ca}^{2+}$  influx and (ii)  $\text{Ca}^{2+}$  influx by CCE is potentiated in cultured cells deficient in PS1, we hypothesized and tested that increased  $\text{Ca}^{2+}$  entry through CCE would be sufficient to induce LTP in CA1 neurons deficient in PS1 and not in wild-type neurons. This was achieved by carrying out experiments in hippocampal slices from transgenic mice with postnatal, neuron-specific ablation of PS1, denominated PS1(n-/-) (19).

### EXPERIMENTAL PROCEDURES

Transgenic mice with neuron-specific deficiency in PS1, denoted PS1(n-/-), were generated by targeting the PS1 gene in embryonic stem cells to contain *loxP* sites. The resulting mice were crossed with transgenic mice that express Cre recombinase specifically and exclu-

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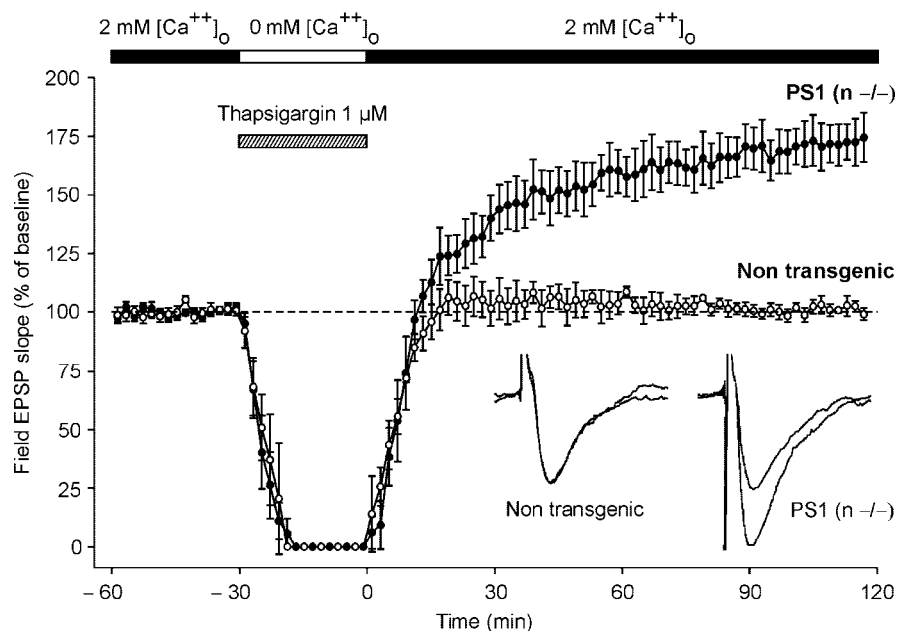
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<sup>1</sup> The abbreviations used are: PS1, presenilin-1; ACSF, artificial cerebrospinal fluid; APV, D(-)-2-amino-5-phosphonovaleric acid; CCE, capacitance calcium entry; ER, endoplasmic reticulum; SERCA, smooth ER  $\text{Ca}^{2+}$ -ATPase pump; EPSP, excitatory postsynaptic potential; fEPSP, field EPSP; LTP, long term potentiation; TG, thapsigargin; PKA, cyclic AMP-dependent protein kinase; JNK, c-Jun NH<sub>2</sub>-terminal kinase; SAPK, stress-activated protein kinase; MAP, mitogen-activated protein; SOC, store-operated calcium channels; VDCC, voltage dependent calcium channels; NMDA, N-methyl-D-aspartate.

**FIG. 1. CCE induces LTP in brain slices from PS1-deficient mice but not in brain slices from non-transgenic mice.** The strength of the synaptic contact that links Schaffer's collaterals to CA1 neurons in hippocampal slices was assessed by the slope of the fEPSP. CCE was induced by incubating slices first in  $\text{Ca}^{2+}$ -free medium containing  $1 \mu\text{M}$  TG for 30 min and subsequently in  $\text{Ca}^{2+}$ -containing medium ( $2 \text{ mM } [\text{Ca}^{2+}]_o$ ). Brain slices from transgenic mice with PS1 inactivation restricted to neurons, *i.e.* PS1(n-/-), were compared with slices from non-transgenic mice. For each mouse type, two representative curves are shown of fEPSPs measured at baseline and 2 h after the TG/ $\text{Ca}^{2+}$ -free treatment. All data points are the mean  $\pm$  S.E. obtained on six slices from the brains of six different mice of each genotype.



sively in their central neurons under the control of the mouse *thy-1* gene promoter (19). The combination of *thy-1-Cre* recombinase and *loxP-PS1* gene was maintained by selection based on four independent PCR reactions performed on DNA extracted from tail-tip cuttings from the offspring. Double transgenic mice were further bred to the homozygous condition for the loxP-modified PS1 gene, to result in a neuron-specific deficiency of PS1. The PS1(n-/-) mice used in the experiments presented here were viable and fertile and have normal brain morphology and normal behavior (19).

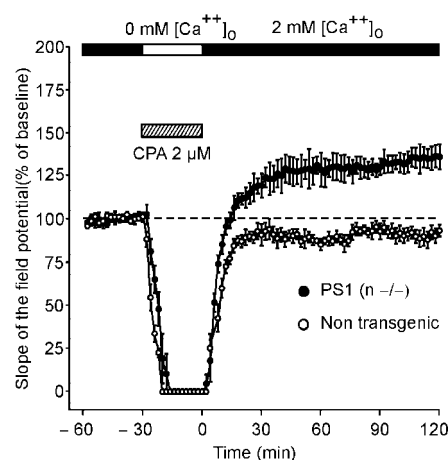
Some experiments were carried out on transgenic mice that express the FAD-mutant PS1(A246). These mice were generated using the *thy-1* gene promoter and have been described and characterized extensively (20).

Hippocampal slices were at all times bathing in artificial cerebrospinal fluid (ACSF) with the following composition: 124 mM NaCl, 5 mM KCl, 26 mM  $\text{NaHCO}_3$ , 1.24 mM  $\text{KH}_2\text{PO}_4$ , 2.4 mM  $\text{CaCl}_2$ , 1.3 mM  $\text{MgSO}_4$ , 10 mM glucose, bubbled with a mixture of 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ . Mice were anesthetized with ether and decapitated. The brain was cut with a vibratome in transverse slices (400  $\mu\text{m}$  thick) in cold ACSF, which were kept afterward at room temperature until placed in a submersion recording chamber at 30  $^\circ\text{C}$ . Electrophysiological recording was started not earlier than 3 h after dissection to allow recovery of the slices. Only a single slice from each tested animal was investigated. The measuring chamber was perfused with ACSF (3 ml/min). Bipolar tungsten microelectrodes (World Precision Instruments, Sarasota, FL) were used to stimulate Schaffer's collaterals, whereas evoked field excitatory postsynaptic potentials were recorded in the stratum radiatum of the CA1 region with low resistance (2 megohm) glass microelectrodes filled with 2 M NaCl. Test stimuli were 0.1-ms constant-voltage pulses delivered every 30 s at an intensity sufficient to evoke an approximate 33% maximal response. The slope of the field excitatory postsynaptic potential (fEPSP, mV/ms) was measured from the average wave from four consecutive responses, as described previously (13, 19, 20). The following commercially available drugs were used: thapsigargin (Sigma), cyclopiazonic acid (Tocris, Bristol, UK), D(-)-2-amino-5-phosphonovaleic acid (Acros), SKF96365 (Tocris), anisomycin (Sigma), and cycloheximide (Sigma).

For each slice, the fEPSP slopes were normalized against the average slope over the 30 min before a specific treatment. To determine whether or not the normalized fEPSP of a group of slices submitted to the same experimental conditions was significantly potentiated ( $p < 0.05$ ), the percentages of baseline measured just before the treatment and 2 h (unless otherwise specified) after its end were compared by a paired Student's *t* test. Statistical significance ( $p < 0.05$ ) of the difference in increase of the fEPSP measured 2 h after a treatment applied to two distinct groups of slices was assessed by a Student's *t* test.

## RESULTS

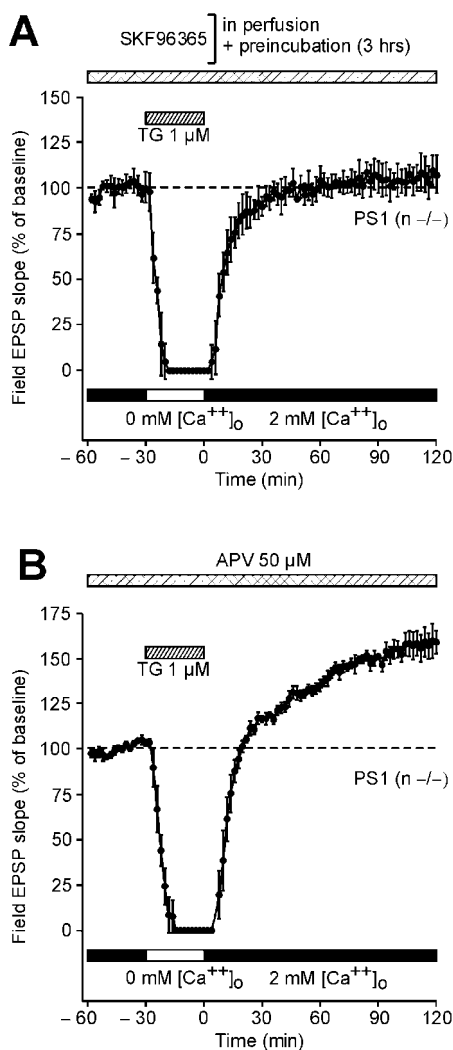
**CCE-induced LTP in Hippocampus of PS1(n-/-) Mice—**Classically, LTP is induced by tetanic stimulation, and it is well



**FIG. 2. Transient application of  $\text{Ca}^{2+}$ -free medium containing cyclopiazonic acid acts as  $\text{Ca}^{2+}$ -free medium containing thapsigargin to induce LTP in brain slices from PS1(n-/-) mice.** CCE was induced by incubating the hippocampal slices to  $\text{Ca}^{2+}$ -free medium containing cyclopiazonic acid (CPA) (2  $\mu\text{M}$ ). Note the enhanced strength of the synapses between Schaffer's collaterals and CA1 neurons in PS1(n-/-) mice but not in non-transgenic mice (see the legend for Fig. 1 for details). All data points are the mean  $\pm$  S.E. obtained on four slices from the brains of four different mice of each genotype.

known that entry of calcium ions is an absolute requirement. Here, we tested the hypothesis that LTP could be induced in PS1-deficient neurons by their overactive mechanism of CCE, *i.e.* the mechanism of replenishing ER  $\text{Ca}^{2+}$  stores. CCE was triggered by first incubating the brain slices in  $\text{Ca}^{2+}$ -free medium containing 1  $\mu\text{M}$  TG, which is known to decrease the  $\text{Ca}^{2+}$  stores of the ER, and subsequently exposing them to normal  $\text{Ca}^{2+}$ -containing medium (Fig. 1). The efficacy of transmission at the synapses between Schaffer's collaterals and CA1 pyramidal cells of the hippocampus was measured with extracellular microelectrodes in the classical manner.

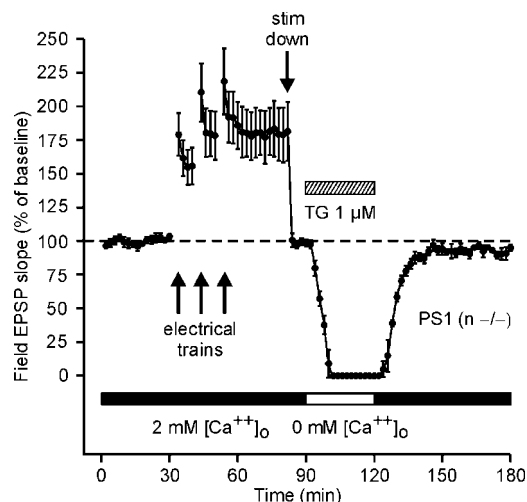
In slices of wild-type mice,  $\text{Ca}^{2+}$ -free perfusion medium resulted in a complete synaptic blockage, evidently by blocking all neurotransmitter release. Upon reperfusion of non-transgenic slices with  $\text{Ca}^{2+}$ -containing medium, the slope of the fEPSP began to recover after about 10 min and subsequently stabilized at control levels over the entire observation period (Fig. 1). After 2 h, the slope of the fEPSP was  $96.5 \pm 9.7\%$



**FIG. 3. LTP induced in brain slices from PS1(n-/-) by transient application of  $\text{Ca}^{2+}$ -free medium depends on CCE and not on the NMDA receptor.** The protocol used to induce LTP was exactly as described in the legend for Fig. 1. In A, SKF96365 (30  $\mu\text{M}$ ) abolished LTP induced by transient application of  $\text{Ca}^{2+}$ -free medium containing thapsigargin, when applied as indicated by the horizontal bar after a preincubation for 3 h. All data points are the mean  $\pm$  S.E. obtained on four slices from the brains of four different PS1(n-/-) mice. In B, APV (50  $\mu\text{M}$ ) did not diminish LTP induced by transient application of  $\text{Ca}^{2+}$ -free medium containing thapsigargin.

(mean  $\pm$  S.D.,  $n = 6$ ), *i.e.* practically unchanged relative to the resting values. The very small variation on these measurements demonstrated the extremely reproducible nature of the response in the six independent control mice analyzed (Fig. 1).

In contrast, this protocol of induction of CCE caused a dramatic increase in the slope of the fEPSP in hippocampal slices from PS1(n-/-) mice (Fig. 1). Two h after stopping the perfusion with TG/ $\text{Ca}^{2+}$ -free medium, the slope of the field EPSP was increased to  $174.8 \pm 18.7\%$  ( $n = 6$ ) of the basal value ( $p < 0.001$ ). This effect was not unique for TG since another agent known to block ER-based calcium pumps and thereby to deplete the ER  $\text{Ca}^{2+}$  stores, *i.e.* cyclopiazonic acid, produced qualitatively the same effect (Fig. 2). Two h after stopping superfusion of the slices with 2  $\mu\text{M}$  cyclopiazonic acid in calcium-free medium, the slope of the fEPSP was also markedly enhanced in CA1 neurons in sections from PS1(n-/-) mice ( $135.0 \pm 12.6\%$ ,  $n = 4$ ,  $p < 0.05$ ) but did not increase at all in slices derived from non-transgenic mice ( $93.2 \pm 6.0\%$ ,  $n = 4$ ) (Fig. 2). These results demonstrated that a protocol known to induce an entry of  $\text{Ca}^{2+}$



**FIG. 4. Occlusion of CCE-induced LTP by tetanus-induced LTP in PS1(n-/-) mice.** LTP was first induced by 3 trains of electric pulses (100 Hz, 1 s), 10 min apart. Stimulation intensity was then reduced to reset the slope of fEPSP at basal levels. Subsequent exposure to TG/ $\text{Ca}^{2+}$ -free medium for 30 min did not enhance synaptic strength.

through the CCE mechanism was able to trigger an LTP in CA1 neurons deficient in PS1, but not in normal ones.

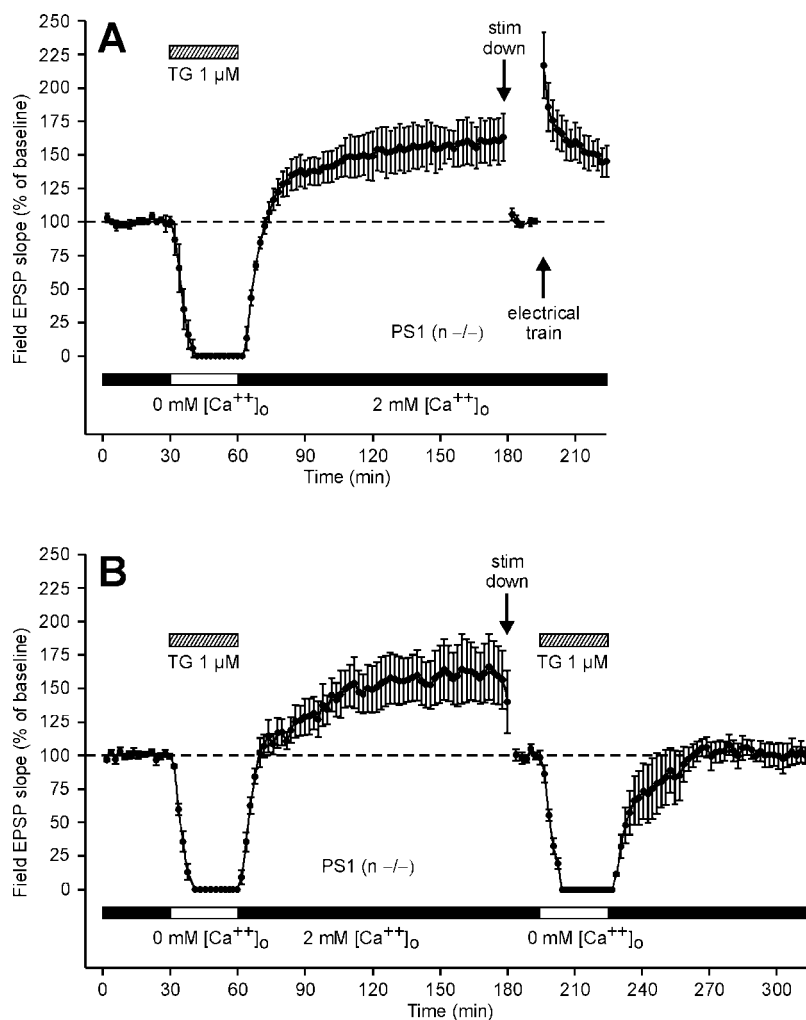
**Origin of  $\text{Ca}^{2+}$  Influx—**LTP induced by incubation in TG/ $\text{Ca}^{2+}$ -free medium was completely blocked by the CCE inhibitor SKF96365 (10) when the slices were pretreated for 3 h with 30  $\mu\text{M}$  (relative change *versus* baseline:  $107.8 \pm 10.5\%$ ,  $n = 4$ ,  $p = 0.12$ ) (Fig. 3A). Because tetanus-induced LTP in the hippocampal CA1 neurons is triggered by an entry of  $\text{Ca}^{2+}$  through the NMDA receptors, we also investigated a potential role of these channels on the TG/ $\text{Ca}^{2+}$ -free induced LTP (Fig. 3B). Brain slices from PS1(n-/-) mice were submitted to the TG/ $\text{Ca}^{2+}$ -free protocol in the presence of 50  $\mu\text{M}$  APV, a well known antagonist of the NMDA receptor. APV modified neither the profile of the CCE-induced LTP nor its amplitude measured 2 h after application of TG/ $\text{Ca}^{2+}$ -free treatment. The values of the fEPSP were  $158.9 \pm 11.0\%$  ( $n = 4$ ) in the presence of APV (Fig. 3B) *versus*  $174.8 \pm 18.7\%$  ( $n = 6$ ) in the absence of APV (Fig. 1,  $p = 0.16$ ).

**Relationship between CCE-induced LTP and Tetanus-induced LTP—**Tetanus-evoked LTP occluded the induction of TG/ $\text{Ca}^{2+}$ -free induced LTP (Fig. 4). Thirty min after the induction of strong LTP by three tetanic stimulations (1 s, 100 Hz) separated from each other by 10 min, the stimulation intensity was reduced to obtain fEPSP with amplitudes corresponding to the control values. Ten min later, the slice was submitted to the  $\text{Ca}^{2+}$ -free medium containing 1  $\mu\text{M}$  TG for 30 min. In this situation, subsequent TG/ $\text{Ca}^{2+}$ -free treatment did not induce LTP since after 1 h, the fEPSP was measured to be  $95.2 \pm 4.1\%$  ( $n = 4$ ) relative to basal values ( $p = 0.50$ ).

However, the converse was not true since the LTP induced by TG/ $\text{Ca}^{2+}$ -free medium did not occlude subsequent tetanus-evoked LTP (Fig. 5A). Two h after the induction of LTP by TG/ $\text{Ca}^{2+}$ -free treatment, the stimulation intensity was reduced to obtain fEPSP with amplitudes corresponding to the control values for 15 min. A single tetanus (1 s, 100 Hz) applied at that moment evoked LTP, *i.e.* 30 min after the application of the tetanus, fEPSP was  $145.3 \pm 20.4\%$  of control values ( $n = 4$ ,  $p < 0.05$ ).

To rule out the possibility that the observed absence of occlusion of tetanus-induced LTP by CCE-induced LTP (Fig. 5A) was due to the fact that the CCE-induced LTP was not saturated yet at the moment of the application of the electrical tetanus, the following control experiment was performed (Fig.

**FIG. 5. Lack of occlusion of tetanus-induced LTP by CCE-induced LTP in PS1(n-/-) mice.** **A**, LTP was first induced by TG/Ca<sup>2+</sup>-free medium for 30 min. Two h later, the intensity of stimulation was decreased to reset the slope of fEPSP to baseline levels. Subsequently, a single tetanic stimulus (100 Hz, 1 s) elicited a significant increase in synaptic strength for at least 30 min. All data points are the mean  $\pm$  S.E. obtained on four slices from the brains of four different PS1(n-/-) mice. **B**, control experiment demonstrating that CCE-induced LTP was saturated at the moment tetanic stimulus was applied in the experiment shown in **A**. The first TG/Ca<sup>2+</sup>-free treatment induced an increase in synaptic strength similar to that induced in **A**. Two h after the end of the first TG/Ca<sup>2+</sup>-free treatment, the intensity of stimulation was decreased to reset the slope of fEPSP to baseline levels. Subsequently, a second TG/Ca<sup>2+</sup>-free treatment was applied that did not elicit significant increase in synaptic strength for the next 2 h. All data points are the mean  $\pm$  S.E. obtained on four slices from the brain of four different PS1(n-/-) mice.



5B). Two h after the induction of LTP by TG/Ca<sup>2+</sup>-free treatment, the stimulation intensity was reduced to obtain fEPSP amplitudes corresponding to baseline for 15 min. A second TG/Ca<sup>2+</sup>-free treatment applied at that time did not induce any LTP since 90 min after the second stimulus, the fEPSP was measured to be  $97.9 \pm 5.5\%$  ( $n = 4$ ) relative to basal values ( $p = 0.35$ ).

**CCE-induced LTP and Protein Synthesis**—To investigate whether CCE-induced LTP was dependent on protein synthesis, we applied anisomycin, an inhibitor of protein synthesis, for 30 min before, during (30 min), and for 30 min after perfusion of the slices with a Ca<sup>2+</sup>-free medium containing thapsigargin. Pretreatment with anisomycin completely abolished the synaptic enhancement induced by TG/Ca<sup>2+</sup>-free treatment in PS1(n-/-) mice ( $98.3 \pm 15.6$ ,  $n = 4$ ,  $p = 0.94$ ) (Fig. 6A).

To demonstrate that the effect of anisomycin was related to inhibition of protein synthesis and not to another effect of the drug, which is also a potent activator of stress-activated protein kinases (JNK/SAPK) and p38 MAP kinase, we tested the effect of cycloheximide, an unrelated inhibitor of protein synthesis, at a concentration of 60  $\mu$ M according to the same temporal pattern as anisomycin. Cycloheximide also nearly completely blocked CCE-induced LTP in PS1(n-/-) mice (Fig. 6B). Two h after the TG/Ca<sup>2+</sup>-free treatment, the fEPSP was as low as  $113.7 \pm 13.5\%$  ( $n = 5$ ) relative to basal values ( $p = 0.34$ ). We conclude that CCE-induced LTP was dependent on protein synthesis.

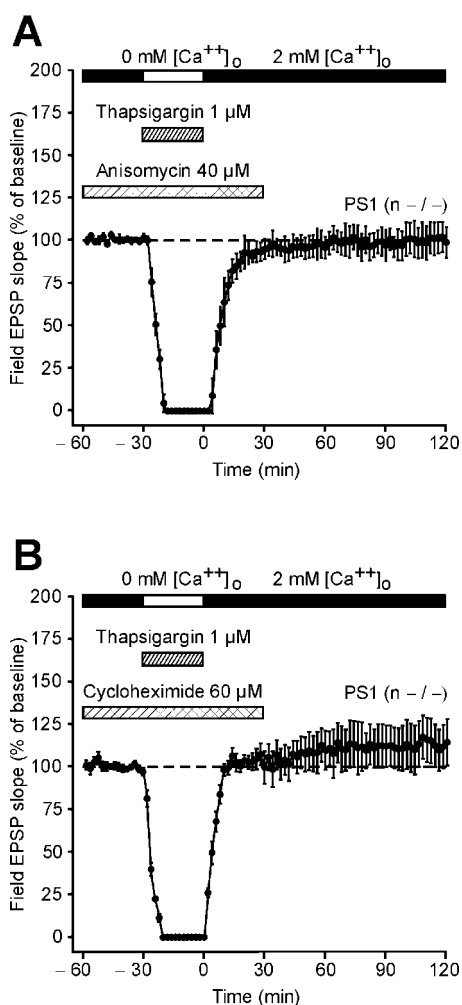
**Mutant PS1 and CCE-induced LTP**—Cultured neurons and fibroblasts harboring a mutant PS1 causing familial Alzheimer's

disease have markedly reduced CCE (10, 11). We therefore predicted that the protocol based on TG/Ca<sup>2+</sup>-free triggering would not produce LTP in CA1 neurons in brain slices from mutant PS1 mice. This prediction was proven in slices from the brain of transgenic mice that express the early onset cases of familial Alzheimer's disease mutant PS1(A246E) (13). Indeed, the TG/Ca<sup>2+</sup>-free medium was unable to induce significant hippocampal LTP in PS1(A246E) slices since after 2 h of TG/Ca<sup>2+</sup>-free perfusion, the slope of the fEPSP was  $99.2 \pm 22.4\%$  ( $n = 5$ ,  $p = 0.49$ ) (Fig. 7).

## DISCUSSION

The major finding of this work is the following. The treatment applied to the hippocampal slices, which is Ca<sup>2+</sup>-free/thapsigargin followed by normal ACSF, induced an LTP of the synaptic strength between Schaffer's collaterals and CA1 neurons in PS1(n-/-) mice but not in wild-type mice. Such an LTP was due to Ca<sup>2+</sup> entry by the CCE mechanism, as it was blocked by an agent known to block CCE (SKF96365). This is thus experimental evidence obtained on brain slices where neuronal networks were intact, and not based on isolated or cultured neurons, for a role of presenilin 1 in the refilling mechanism of the intracellular Ca<sup>2+</sup> stores.

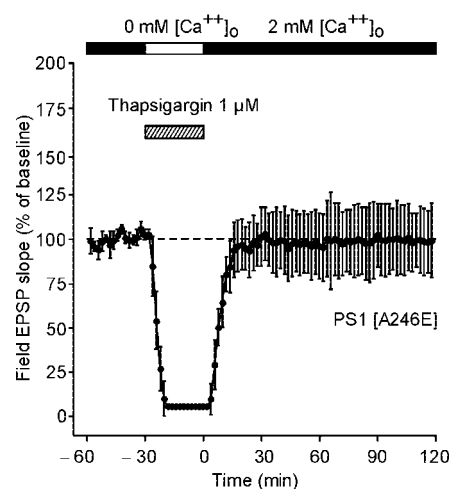
Before discussing the relationships between CCE-induced LTP and tetanus-induced LTP, it is worth remembering the characteristics of the classical tetanus-induced LTP whose major actors are shown below (see Fig. 8). LTP caused by multiple trains of high frequency electrical stimulation can be divided into two phases: (i) an early phase, consisting of an induction



**FIG. 6. CCE-induced LTP in PS1(n<sup>-/-</sup>) hippocampal slices is dependent on protein synthesis.** Inhibition of protein synthesis both by anisomycin (40 μM) (A) and by cycloheximide (60 μM) (B) suppressed CCE-induced LTP in hippocampal slices from PS1(n<sup>-/-</sup>) mice (compare with Fig. 1). The respective timings of applications of anisomycin (or cycloheximide) and Ca<sup>2+</sup>-free medium containing thapsigargin are indicated by labeled bars. All data points are the mean ± S.E. obtained on four slices from four different PS1(n<sup>-/-</sup>) mice in A and on five slices from five different PS1(n<sup>-/-</sup>) in B.

mechanism and an early maintenance phase; and (ii) a late phase, which is a maintenance phase (21). Induction is triggered by initial increase of cytosolic Ca<sup>2+</sup> in dendritic spines due mainly to Ca<sup>2+</sup> entry through NMDA receptors (22). In this induction phase, activation of calcium/calmodulin-dependent protein kinase (23) but also of protein kinase C (24) are believed to play a pivotal role. In contrast to the early phase, the late maintenance phase of tetanus-induced LTP requires new protein synthesis (25–27). This is triggered by a cascade initiated by still another kinase, the cyclic AMP-dependent protein kinase (PKA), which is activated by the large Ca<sup>2+</sup> influx provoked by repeated trains of action potentials (21, 28).

All the signaling pathways underlying the tetanus-induced LTP (both early and late phases) are unaffected by genetic ablation of PS1 (19, 29). In PS1(n<sup>-/-</sup>) mice, calcium entering the neurons through the CCE mechanism triggers long term potentiation by activating only pathway(s) involved in tetanus-induced LTP (since tetanus-induced LTP occludes CCE-induced LTP), although not all of them (since CCE-induced LTP does not occlude tetanus-induced LTP). One of the shared pathways is that leading to the late phase of tetanus-induced LTP. Indeed, CCE-induced LTP and the late phase of

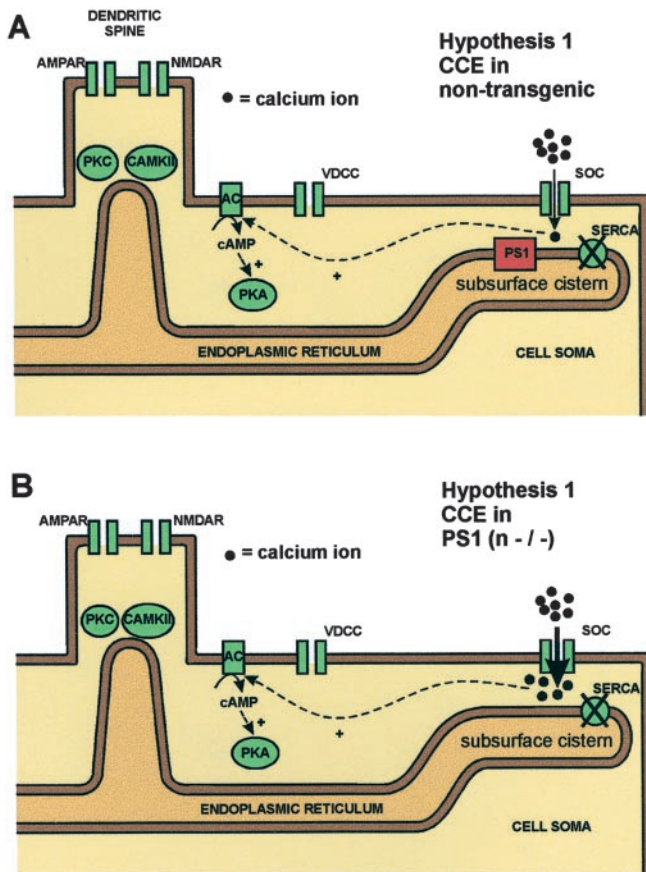


**FIG. 7. CCE did not induce LTP in hippocampal slices from mutant PS1(A246E) transgenic mice.** The same protocol used for PS1(n<sup>-/-</sup>) mice (see “Experimental Procedures” and Fig. 1) failed to induce any significant change in the slope of fEPSP after TG/Ca<sup>2+</sup>-free treatment. All data points are the mean ± S.E. obtained on five slices from the brains of five different PS1(A246E) transgenic mice.

tetanus-induced LTP share the property of being protein synthesis-dependent.

PS1 is a serpentine protein known to be localized mainly in the ER. At the postsynaptic level, it is not clear whether the changes of CCE induced by a PS1 deficiency take place in the dendritic spines, in the dendritic shafts, and/or in the cell soma. Whereas the presence of functional ER calcium stores in the spines of hippocampal CA1 is attested by the fact that ryanodine receptors are enriched in dendritic spines (30), subsurface cisterns of ER are closely apposed to the plasma membrane of the soma (14). It is clear that Ca<sup>2+</sup> entering the dendritic spines by the CCE mechanism could have access to the signaling pathways of the tetanus-induced LTP. However, even if the increased CCE in PS1(n<sup>-/-</sup>) mice was confined to the soma, it could be sufficient to activate the late maintenance component of the tetanus-induced LTP. Indeed, APV blocks the early phase of LTP but not the late phase of LTP, whereas nifedipine, which blocks L-type voltage-dependent calcium channels (VDCC), attenuates the late phase of LTP (28). This suggests that the late phase of tetanus-induced LTP is triggered by Ca<sup>2+</sup> ions, which enter not only through NMDA receptors but also through L-type VDCC channels. In this regard, it is interesting to note that L-type VDCC are localized in the somatic membrane and proximal dendrites (31). Thus, entry of Ca<sup>2+</sup> through SOC located in the plasma membrane of the soma or of the dendritic spines could be responsible for the induced enhancement of the synaptic strength.

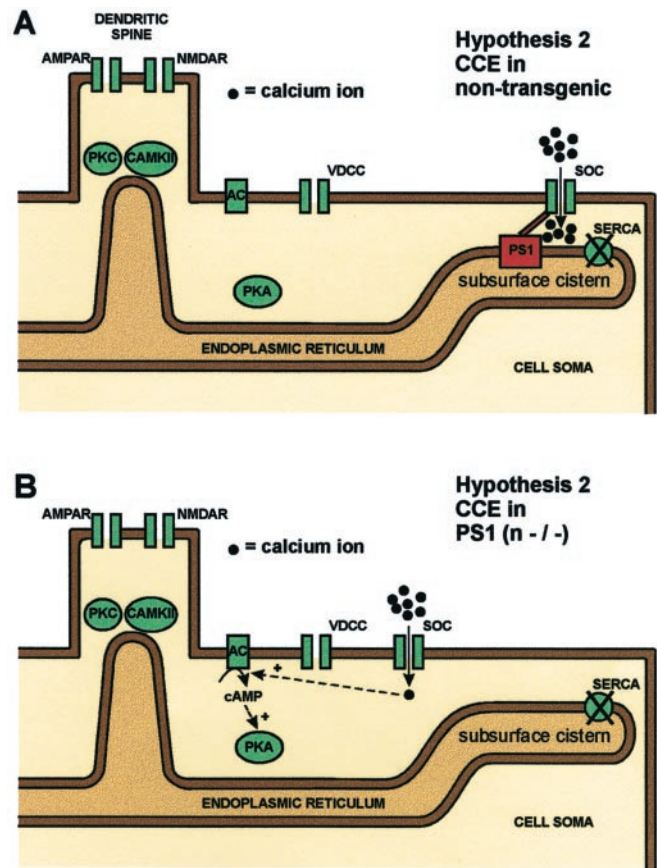
Because functional calcium stores are present not only in the soma and dendrites, but also in synaptic boutons (32, 33), a possible presynaptic mechanism responsible for the CCE-induced LTP in the PS1(n<sup>-/-</sup>) mice deserves further discussion. In synaptic terminals of CA3 pyramidal cells, a presynaptic action potential causes a transient increase in Ca<sup>2+</sup> due primarily to an entry of Ca<sup>2+</sup> from the extracellular space through the VGCC but also to a calcium-induced release of calcium by the presynaptic calcium stores (32). It has been demonstrated that a depletion of the Ca<sup>2+</sup> stores in presynaptic terminals by cyclopiazonic acid or TG induced a decrease in the calcium transients evoked by action potentials in presynaptic boutons. However, as this effect is a decrease and as it does not affect the amplitude of EPSPs caused by single stimuli, it cannot be responsible for the increase in synaptic strength observed in CCE-induced LTP. Pharmacologically induced depletion of



**FIG. 8. First hypothetical model explaining why CCE induces an LTP in PS1(n-/-) mice (B) and not in non-transgenic mice (A).** The major actors involved in electrical tetanus-induced LTP ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (AMPA), NMDA receptors (NMDAR), protein kinase C (PKC), calcium/calmodulin protein kinase II (CaMKII), adenylate cyclase (AC), PKA, and VDCC) are shown together with the agents responsible for the refilling of the  $\text{Ca}^{2+}$  stores in the endoplasmic reticulum (SOC and SERCA). In tetanus-induced LTP, the late phase requires PKA activation, which in turn triggers an appropriate protein synthesis. Notice the continuity of the endoplasmic reticulum, which sends expansions in dendritic spines and forms subsurface cisterns in the soma (14). In our experiments, CCE was induced by submitting the slices first to thapsigargin in  $\text{Ca}^{2+}$ -free medium and afterward to normal ACSF. (A and B). According to hypothesis 1 (LaFerla's hypothesis), PS1 favors the refilling mechanism of the  $\text{Ca}^{2+}$  stores in the ER, and CCE is induced when the calcium stores fall below a threshold level. In non-transgenic mice, calcium ions entering by the CCE mechanism through the SOC would act on adenylate cyclase and hence indirectly activate PKA, but not at a level sufficient to induce a late LTP (A). In PS1(n-/-) mice, the amount of  $\text{Ca}^{2+}$  ions entering by CCE would be increased and reach a level sufficient to induce a robust activation of PKA (B).

$\text{Ca}^{2+}$  stores in synaptic boutons also causes another effect, *i.e.* it activates the store-operated channels of the presynaptic membrane. The resulting continuous influx of calcium through the SOC then increases the frequency of the miniature EPSPs (32). This mechanism, however, is not involved in the CCE-induced LTP either since an increased rate of spontaneous transmitter release would act at the synapses through the NMDA receptors, whereas CCE-induced LTP is not blocked by APV (Fig. 3B)

The next question is how CCE enhances synaptic strength in the absence of PS1 and not in its presence. The first possibility is that the amount of  $\text{Ca}^{2+}$  entering the cell by the CCE mechanism is increased by the absence of PS1 and so sufficient to reach the threshold level needed to trigger the signal cascades leading to an increased synaptic efficacy (Fig. 8, A and B). By measuring the concentration of  $\text{Ca}^{2+}$  in the cytosol of cultured



**FIG. 9. Second hypothetical model explaining why CCE induces an LTP in PS1(n-/-) mice (B) and not in non-transgenic mice (A).** The labeling and definitions are the same as in Fig. 8. In A and B, according to hypothesis 2, PS1 helps to maintain SOC in front of SERCA pumps. A "microdomain" is so formed between the plasma membrane and the membrane of the corresponding subsurface cistern. Following this hypothesis, in non-transgenic mice,  $\text{Ca}^{2+}$  entering the cell is trapped in the vicinity of the subsurface cistern without access to PKA (A). According to that second hypothesis, in PS1(n-/-) mice, the amount of  $\text{Ca}^{2+}$  entering the cell by the CCE mechanism is not increased, but  $\text{Ca}^{2+}$  ions now have access to PKA, as a result of the fact that SOC are no longer maintained in front of the subsurface cisterns (B).

cells, it has been demonstrated that genetic ablation of PS1 resulted in: (i) an increased entry of  $\text{Ca}^{2+}$  by the CCE mechanism (10) and (ii) a decrease in their ER- $\text{Ca}^{2+}$  stores (12). LaFerla's group (11) had proposed previously that CCE was activated when calcium levels within the lumen of the ER fell below a threshold level. According to these authors, CCE would be increased in PS1 knock-out cells because their ER- $\text{Ca}^{2+}$  stores are abnormally low (12).

A second possibility that could explain how CCE induces LTP in PS1 knock-out neurons and not in control neurons is that the absence of PS1 would not modify the amount of  $\text{Ca}^{2+}$  entering by the CCE mechanism, but instead its access to its targets. PS1 could structurally help to position and maintain SOC close to SERCA pumps at the level of the subsurface cisterns of the ER, thus organizing a local "signaling microdomain" (34). In this case,  $\text{Ca}^{2+}$  ions entering through SOC would either be readily pumped into the ER in the absence of thapsigargin or be locally "trapped" when SERCA is blocked irreversibly by thapsigargin (Fig. 9A). In the absence of PS1, clustering of SOC and their apposition in front of subsurface cisterns of ER would be lost, and  $\text{Ca}^{2+}$  entering through SOC would no longer be locally trapped between the membrane of the cell and that of the corresponding subsurface cistern. That would allow  $\text{Ca}^{2+}$  to have direct access to its target the adenylate

cyclase and hence activate PKA and trigger enhanced synaptic strength (Fig. 9B).

A final question, not addressed in this study but most interesting to raise, is whether the increase in CCE observed in PS1-deficient mice is related to their evident inhibition of  $\gamma$ -secretase activity (19). Most recently, the first two effects were claimed to be mimicked by  $\gamma$ -secretase inhibitors (12). On the other hand, it is most interesting to note that PS1 acts on CCE directly, independently of APP, as observed in neurons from APP-null mice (35). It is clear that fundamentally we do not fully understand the diverse functions of PS1 and the context of its dual function in regulated proteolytic activity and in calcium signaling.

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