Biphasic requirement for geranylgeraniol in hippocampal long-term potentiation

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Mice deficient in cholesterol 24-hydroxylase exhibit reduced rates of cholesterol synthesis and other non-sterol isoprenoids that arise from the mevalonate pathway. These metabolic abnormalities, in turn, impair learning in the whole animal and hippocampal long-term potentiation (LTP) *in vitro*. Here, we report pharmacogenetic experiments in hippocampal slices from wild-type and mutant mice that characterize the dependence of LTP on the non-sterol isoprenoid, geranylgeraniol. Addition of geranylgeraniol to slices from 24-hydroxylase knockout mice restores LTP to wild-type levels; however, farnesol, a chemically related compound, does not substitute for geranylgeraniol nor does another animal model of impaired LTP (apolipoprotein E deficiency) respond to this isoprenoid. The requirement for geranylgeraniol is independent of acute protein isoprenylation as judged in experiments employing cell-permeable inhibitors of protein farnesyl transferase and geranylgeranyl transferase enzymes and in mutant mice hypomorphic for geranylgeranyltransferase II. Time course studies show that geranylgeraniol acts within 5 min and at 2 different times during the establishment of LTP: just before electrical stimulation and approximately 15 min thereafter. Localized delivery of geranylgeraniol to the dendritic trees of CA1 hippocampal neurons via the recording electrode is sufficient to restore LTP in slices from 24-hydroxylase knockout mice. We conclude that geranylgeraniol acts specifically and quickly to affect LTP in the Schaffer collaterals of the hippocampus.

brain | cholesterol 24-hydroxylase | cytochrome p450 | learning | memory

A major mechanism by which cholesterol is turned over in the brain involves conversion of the sterol into 24*S*-hydroxycholesterol by the P450 enzyme, cholesterol 24-hydroxylase (1–5). 24*S*-Hydroxycholesterol crosses the membranes of cells in which it is made, gains access to the circulation, and is thereafter cleared by the liver (6, 7). The cholesterol that is turned over in this manner is replaced by the synthesis of new cholesterol so that steady-state levels of the sterol in the brain remain constant (3).

In situ mRNA hybridization and immunohistochemical experiments show that 24-hydroxylase is expressed in neurons but not support cells (5, 8), suggesting a biological function for cholesterol turnover that is independent of myelin formation and maintenance. In agreement with this idea, knockout mice lacking 24-hydroxylase exhibit significant defects in spatial, associative, and motor learning *in vivo*, and impaired hippocampal long-term potentiation (LTP) *in vitro* (9). The LTP phenotype is not caused by obvious anatomical or histological defects in the brains of the knockout mice (8, 9), but rather arises indirectly from the decreased synthesis of new cholesterol that accompanies the reduction in turnover associated with loss of the 24-hydroxylase.

The metabolic pathway that produces cholesterol also synthesizes several non-sterol isoprenoid end-products (Fig. 1), including farnesyl diphosphate and geranylgeranyl diphosphate, whose synthetic rates are similarly reduced in the 24-hydroxylase knockout mice. It is the reduction in geranylgeranyl diphosphate synthesis that causes the LTP defect as the addition of geranylgeraniol, a precursor of geranylgeranyl diphosphate, to hippocampal slices from 24-hydroxylase knockout mice restores LTP to levels found in wild-type slices. Conversely, pharmacologic inhibition of cholesterol synthesis in wild-type slices with a statin causes reduced LTP and this inhibition is prevented by the presence of exogenous geranylgeraniol (9).

In the current study, the dependence of LTP on geranylgeraniol is explored further. We show that the response to this polyisoprenol is selective at both the chemical and genetic levels and that it does not appear to be related to protein isoprenylation. Kinetic studies indicate that geranylgeraniol acts rapidly and is required for both the induction and maintenance of hippocampal LTP.

Results

Geranylgeraniol Restores Hippocampal LTP in 24-Hydroxylase Knockout Mice. The general characteristics of the LTP assay used in this study are illustrated in [supporting information (SI) Fig. S1*A*]. Following theta-burst stimulation, potentiation is induced in hippocampal slices from wild-type mice and is maintained through a subsequent 60 min recording period. This form of synaptic plasticity is commonly referred to as early LTP (10, 11), and in agreement with this designation, the presence of protein synthesis inhibitors such as anisomycin (20 μM) did not affect potentiation elicited with the stimulation protocol used here (T. Kotti, unpublished observations).

Slices from 24-hydroxylase knockout mice (−/−) showed little potentiation (Fig. S1*A*); however, the addition of 0.2 mM geranylgeraniol to the artificial cerebrospinal fluid (ACSF) at time 0 resulted in LTP that was indistinguishable from that in wild-type slices. The addition of 0.2 mM farnesol, a precursor of geranylgeraniol, also restored LTP in knockout slices but did not do so if a statin was included in the buffer (Fig. S1*B*). Statins inhibit the synthesis of mevalonate, a precursor of isopentenyl diphosphate, which in turn is required to elongate farnesyl diphosphate to geranylgeranyl diphosphate (Fig. 1).

To determine whether geranylgeraniol would restore LTP in a different genetic model of impaired LTP, a series of experiments was performed using hippocampal slices from apolipoprotein E (apoE)-deficient mice. ApoE is a ligand for members of the low density lipoprotein receptor family with important functions in cholesterol metabolism, cognition, and neurodegenerative disease (12, 13). We confirmed an earlier report that apoE-deficient mice exhibit defects in hippocampal LTP (14); however, supplementation of the ACSF with 0.2 mM geranylge-

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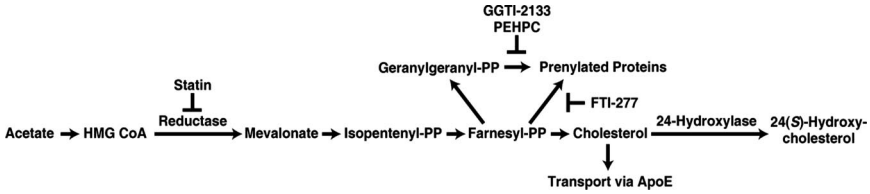

Fig. 1. Schematic of cholesterol synthesis and turnover in the brain showing selected pathway intermediates and inhibitors used in this study. Statins inhibit HMG CoA reductase early in the biosynthetic pathway, whereas FTI-277, GGTI-2133, and 3-PEHPC block the addition of isoprenyl groups to protein targets. The 24-hydroxylase turns over cholesterol by converting it to 24(*S*)-hydroxycholesterol, a membrane-permeable end-product. Synthesis of new cholesterol to replace that turned over ensures a constant flow of intermediates through the pathway, one of which, geranylgeranyl diphosphate or a derivative of this polyisoprenoid, is required for LTP. Apolipoprotein E (*ApoE*) facilitates the movement of cholesterol between different cell types, either as a component of lipoprotein particles or in the form of ApoE-cholesterol complexes.

raniol did not restore LTP in these slices (Fig. S1*C*). Together, the data presented in Fig. S1 suggested that the ability of geranylgeraniol to restore LTP in 24-hydroxylase deficient mice was chemically and genetically specific.

Short-Term Treatments with Inhibitors of Protein Isoprenylation Do Not Affect LTP. Synaptic transmission requires the participation of numerous proteins that must be posttranslationally modified by the covalent addition of farnesyl or geranylgeranyl for activity (15). Effective cell-permeable inhibitors of the protein farnesyltransferase (E.C. 2.5.1.58) and the protein geranylgeranyltransferase I (E.C. 2.5.1.59) and II (E.C. 2.5.1.60) that catalyze these reactions are available and were used to determine whether the requirement for geranylgeraniol in LTP reflected a need for protein isoprenylation. The data of Fig. S2*A* show that when a selective inhibitor (FTI-277) of farnesyltransferase was added to the ACSF, LTP was induced normally in hippocampal slices from wild-type mice. Similarly, inclusion of a selective inhibitor (GGTI-2133) of geranylgeranyltransferase I for the duration of the 80-min experiment did not impair LTP (Fig. S2*B*). As expected, supplementation of ACSF with both the geranylgeranyltransferase I inhibitor and 0.2 mM geranylgeraniol did not affect the ability of the polyisoprenol to restore LTP in 24-hydroxylase knockout slices (Fig. S2*C*).

Two types of experiments were done to test the role of geranylgeranyltransferase II in LTP. This enzyme is also referred to as the Rab geranylgeranyltransferase. First, hippocampal LTP was measured in *gunmetal* mice, which have an unusual gray coat color and express reduced amounts of geranylgeranyltransferase II activity (approximately 30% of normal) as a consequence of a point mutation that disrupts splicing of a majority of mRNAs transcribed from the gene encoding the α-subunit of this heterodimeric enzyme (16). As shown in Fig. 2*A*, LTP was normal in slices from mice homozygous for the *gunmetal* allele. The addition of the geranylgeranyltransferase I inhibitor to the ACSF at time 0 did not reduce LTP in *gunmetal* slices (Fig. 2*B*), nor was LTP affected by a specific inhibitor (3-PEHPC) of geranylgeranyltransferase II (Fig. 2*C*). To determine whether geranylgeraniol was required for LTP in *gunmetal* slices, a statin was included in the ACSF from time 0 onwards to inhibit the synthesis of endogenous geranylgeraniol. LTP was reduced in the presence of the statin alone, and this inhibition was prevented by coaddition of the statin plus exogenous geranylgeraniol (Fig. 2*D*).

Long-Term Treatments with Inhibitors of Protein Isoprenylation Decrease LTP. As controls for the above experiments, we incubated hippocampal slices from wild-type mice for extended periods of time (3 to 7 h, see *Materials and Methods*) in each of the 3 protein isoprenylation inhibitors before measuring LTP. Long-term incubation of tissue in the presence of the farnesyltransferase inhibitor reduced LTP (Fig. S3*A*), whereas in the presence of the geranylgeranyltransferase I inhibitor, LTP was induced normally but declined to baseline over a 60-min period (Fig. S3*B*). The geranylgeranyltransferase II inhibitor 3-PEHPC was less effective at reducing LTP but an impairment of potentiation was evident (Fig. S3*C*). Under the assumption that these inhibitors diffuse into brain cells as quickly as they do other cells, we concluded from the pharmacologic and genetic experiments shown in Fig. 2 and Figs. S2 and S3 that protein isoprenylation was important for LTP but not in the acute time frame during which geranylgeraniol was acting.

Phenotypes in Mice Heterozygous for the 24-Hydroxylase Mutation. We next examined the phenotype of mice heterozygous for the 24-hydroxylase gene mutation. As shown by the data in Fig. 3*A*, compared to the wild-type and homozygote responses, hippocampal slices from heterozygous mice showed a different LTP phenotype in which potentiation was initiated normally but declined to baseline between min 40 to 60 of the experiment. As in homozygous tissue, inclusion of geranylgeraniol in the ACSF restored LTP in heterozygous slices (Fig. 3*B*).

The abnormal LTP response suggested that heterozygous mice might exhibit a phenotype in behavioral learning assays. To explore this possibility, spatial learning was assessed in animals of different 24-hydroxylase genotypes using the Morris water maze test (Fig. 3*C*). Wild-type mice quickly learned the location of the submerged platform in these experiments, and as reported in a previous study (9), homozygous mice were markedly deficient in the task. Heterozygous mice exhibited a phenotype that was intermediate between those of wild-type and homozygous mice. After removal of the platform on various trial days, the number of times heterozygous animals swam across the former location of the platform were also intermediate between those of wild-type and homozygous mice (Fig. 3*D*). In control experiments not shown, heterozygotes swam normally and were not blind (see *SI Materials and Methods*).

Brief Exposure to Geranylgeraniol or Statin Affects LTP. LTP is thought to consist of distinct induction and maintenance phases that are established by different biochemical mechanisms (10, 11). From the data presented in Fig. 3*A*, the LTP phenotype of 24-hydroxylase homozygotes suggested that potentiation was not being induced whereas the phenotype of heterozygotes suggested that potentiation was induced but not maintained. These responses in turn implied that geranylgeraniol was required at two different times to establish LTP. To test this implication, geranylgeraniol was administered to heterozygous slices at different times during an LTP experiment. The data of Fig. 4*A* show that a 5 min delivery of geranylgeraniol (between min 35–40) just before the time when potentiation would normally begin to decline was sufficient to maintain potentiation. If the polyisoprenol was administered after potentiation had begun to decline

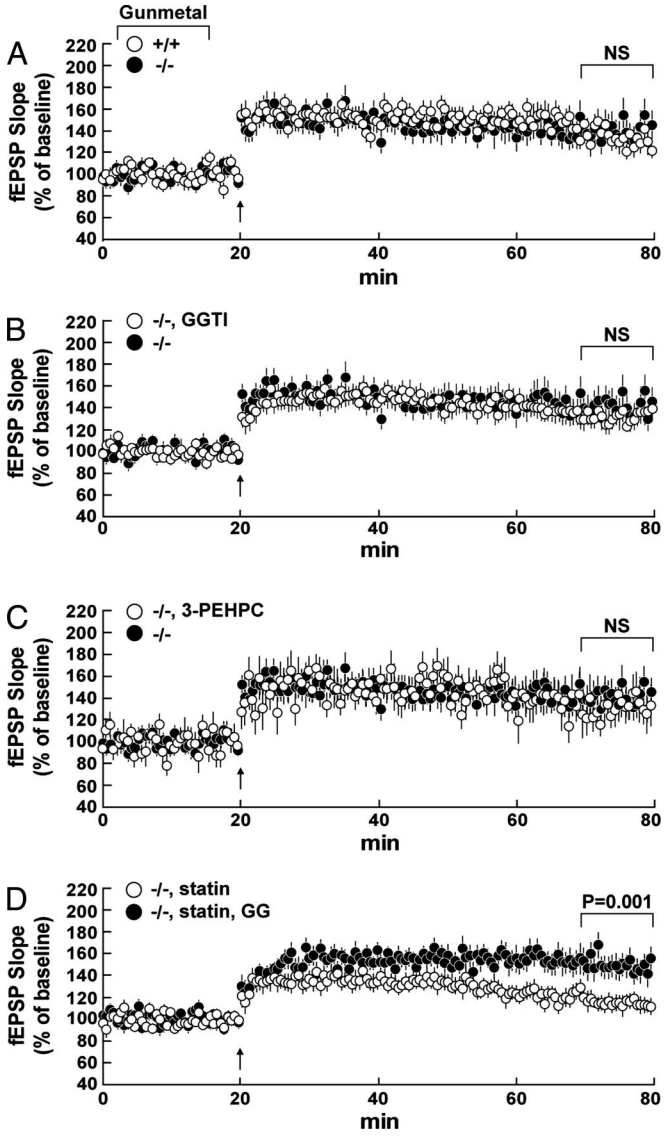

Fig. 2. Protein isoprenylation inhibitors do not reduce LTP in hippocampal slices from *gunmetal* mice that are hypomorphic for geranylgeranyltransferase II. (*A*) LTP in slices from *gunmetal* mice (●, $n = 20$) was indistinguishable from that in wild-type slices (○, $n = 19$). (*B*) Treatment of hippocampal slices from *gunmetal* mice with ACSF alone (●, $n = 20$) or ACSF containing 10 μM GGTI-2133 (○, $n = 19$), an inhibitor of geranylgeranyltransferase I (*GGTI*), did not reduce LTP. Inhibitor administration started at time 0 of the experiment. (*C*) Treatment of hippocampal slices from *gunmetal* mice with ACSF alone (●, $n = 20$) or ACSF containing 1.5 mM 3-PEHPC (○, $n = 18$), an inhibitor of geranylgeranyltransferase II, did not reduce LTP. (*D*) Treatment of hippocampal slices from *gunmetal* mice with an inhibitor of HMG CoA reductase (statin, 12.5 μM) reduced LTP (○, $n = 25$); this inhibition was prevented by inclusion of 0.2 mM geranylgeraniol in the ACSF (●, $n = 21$).

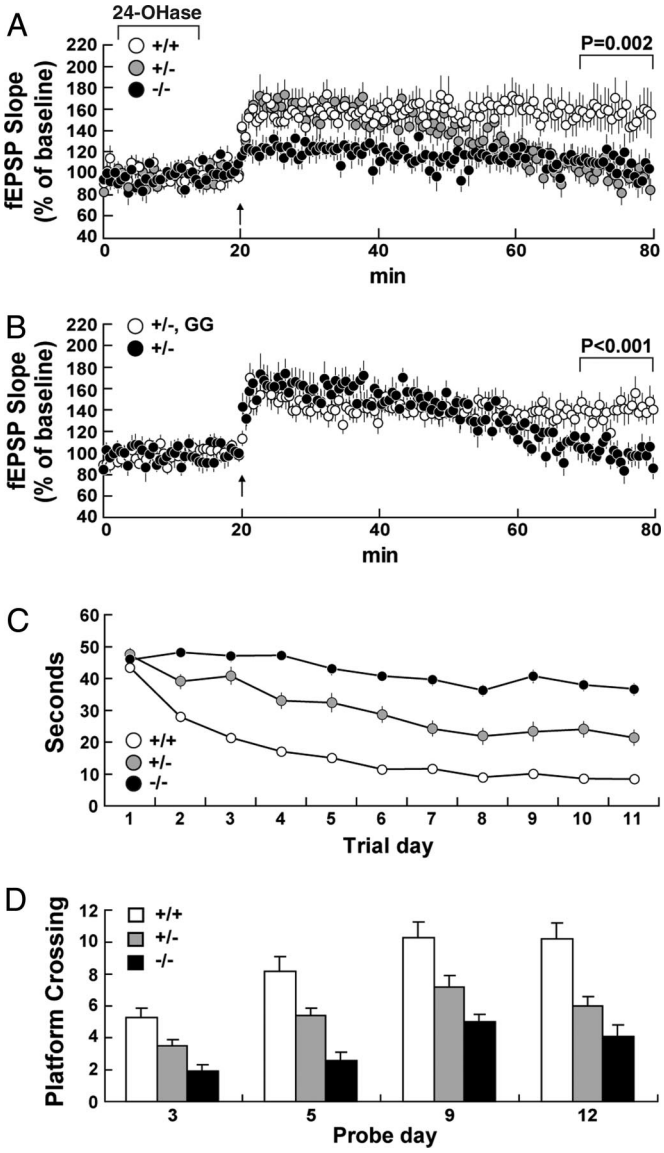

Fig. 3. Mice heterozygous for an induced mutation in the 24-hydroxylase gene show an intermediate phenotype in LTP and spatial learning assays. (*A*) LTP was induced by theta-burst stimulation in hippocampal slices from mice of the indicated 24-hydroxylase genotypes. Slices from animals homozygous for the mutation (−/− , ●, $n = 14$) showed reduced LTP compared to those from wild-type animals (+/+, ○, $n = 18$), while slices from heterozygotes (+/−, shaded circles, $n = 14$) showed an intermediate phenotype in which LTP was induced normally at tetanization but began to decline at approximately 40 min after the start of the experiment and by approximately 60 min reached the level of potentiation observed in homozygous slices. (*B*) Inclusion of 0.2 mM geranylgeraniol (*GG*) in the ACSF throughout the experiment restored LTP in heterozygous slices (○, $n = 12$). (*C*) Spatial learning in mice of the indicated 24-hydroxylase genotypes was assessed in Morris water maze tests. Mean time to find a submerged platform (latency) is shown as a function of trial day. Data were collected in four different experiments involving 40 +/+, 20 +/−, and 30 −/− male mice. Results from the wild-type and homozygous mutant animals were reported previously (9), and are reproduced here for comparison purposes. (*D*) Probe trial responses. The submerged platform was removed from the tank on the indicated days of the Morris water maze experiments and the number of times animals of different 24-hydroxylase genotypes swam across the former location of the platform was recorded. Differences between wild-type and heterozygous mice were statistically significant ($P < 0.05$) on each of the four probe days.

(between min 50–55), then LTP was not maintained and potentiation decreased to baseline (Fig. 4*B*).

We next tested the effects on LTP arising from brief administration of statin to wild-type slices. As shown in Fig. 4*C*, if the statin was administered between min 30–45, then LTP was induced but not maintained and the overall response kinetics were similar to those observed in unsupplemented heterozygous slices (compare Fig. 4*A* to 4*C*). Shorter statin-administration times after theta-burst stimulation did not affect LTP; however, delivery of the drug for a 5 min period spanning the high-frequency stimulation event (between min 17–22) prevented the induction of LTP (Fig. 4*D*) and produced a response that was similar to that observed in un-supplemented homozygous slices.

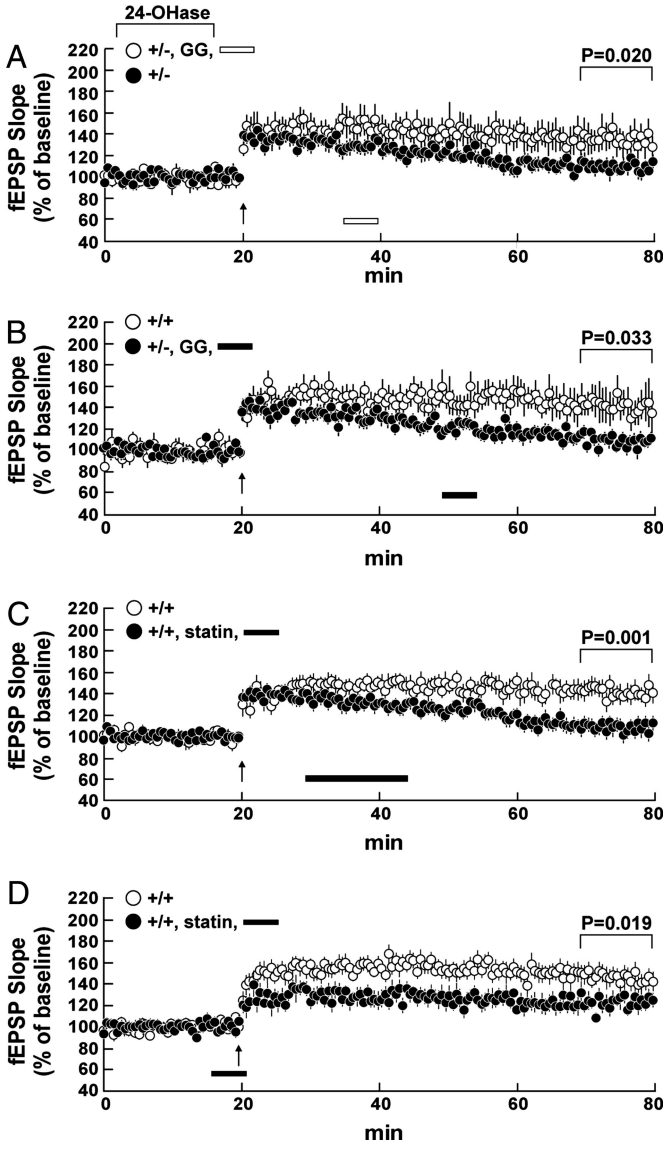

Fig. 4. Brief treatment with geranylgeraniol restores LTP in hippocampal slices from 24-hydroxylase heterozygous mice. (*A*) Slices from heterozygous mice (+/−) were treated with ACSF (●, $n = 14$) or ACSF supplemented with geranylgeraniol (*GG*) (○, $n = 9$) for the 5 min interval (time = 35–40 min) marked by the *open rectangle*. The transient presence of geranylgeraniol prevented the decline in potentiation observed between 40 and 50 min in heterozygous slices treated with ACSF alone. (*B*) Geranylgeraniol treatment delivered between time = 50–55 min did not maintain LTP in heterozygous slices (●, $n = 11$). Data from an experiment with wild-type slices (+/+, $n = 15$) are shown for comparison purposes. (*C*) Hippocampal slices from wild-type mice (+/+) were treated with ACSF alone (○, $n = 12$) or ACSF supplemented with an HMG CoA reductase inhibitor (*statin*, ●, $n = 18$) for the 15 min interval (time = 30–45 min) marked by the closed rectangle. The presence of the drug during this interval caused a decline in potentiation. (*D*) Slices (●, $n = 11$) from wild-type mice were treated with statin before, during, and after high-frequency stimulation (black rectangle, time = 17–22 min). The presence of the inhibitor during this period prevented the initiation of LTP.

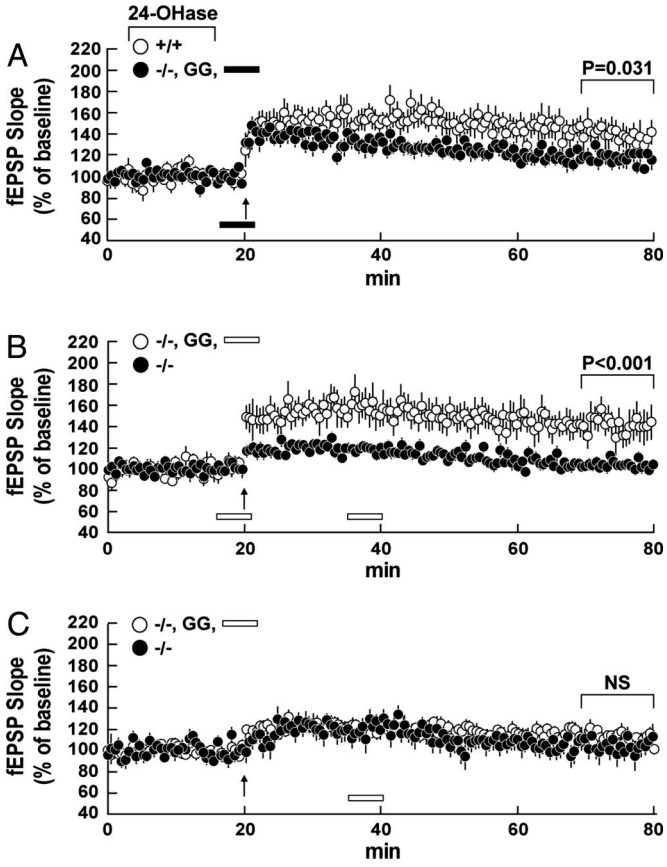

Fig. 5. Brief treatment with geranylgeraniol restores LTP in hippocampal slices from 24-hydroxylase homozygous mice. (*A*) Slices from homozygous mice (−/−) were treated for 5 min with geranylgeraniol (*GG*) during tetanization (●, $n = 13$, closed rectangle, time = 17–22 min). Potentiation was induced transiently in slices by this treatment but decayed over the course of the experiment versus that measured in wild-type slices (○, $n = 8$). The pattern observed resembled that measured in slices from untreated heterozygous mice (e.g., Fig. 4*A*). (*B*) Treatment of hippocampal slices (○, $n = 10$) from knockout mice with geranylgeraniol during stimulation and at time = 35–40 min produces a wild-type level of LTP. Little potentiation was observed in untreated slices (●, $n = 18$). (*C*) Geranylgeraniol treatment at time = 35–40 min does not affect potentiation in knockout slices (○, $n = 11$) versus ACSF-alone treated controls (●, $n = 17$).

Fig. 5 shows data from experiments of similar design that used slices from 24-hydroxylase knockout mice. Administration of geranylgeraniol between min 17–22 induced LTP but did not maintain it (Fig. 5*A*); however, delivery between min 17–22 and min 35–40 induced and maintained LTP (Fig. 5*B*). As expected, providing geranylgeraniol in the 35–40 min interval alone did not induce potentiation (Fig. 5*C*). The data presented in Figs. 4 and 5 supported the idea that geranylgeraniol was required at two different times for LTP; first, before or during the delivery of high frequency stimulation to induce potentiation, and second, between min 35–40 to maintain potentiation. These data also showed that administration of statin between min 17–22 to wild-type slices produced a longer lasting effect than achieved with geranylgeraniol in knockout slices as potentiation did not change during the course of the drug experiment (compare Fig. 4*D* to Fig. 5*A*).

Localized Delivery of Geranylgeraniol or Statin Affect LTP. In the experiments reported above, prenyltransferase inhibitors, geranylgeraniol, or statin were administered in the ACSF that was circulated past hippocampal slices during the recording of field potentials. To determine whether localized delivery of these agents would modulate LTP, we included 10 μM FTI-277, 10 μM GGTI-2133, 0.2 mM geranylgeraniol, or 12.5 μM statin in the buffer of the recording electrode and then measured LTP. Delivery of geranylgeraniol in this manner increased LTP in knockout slices to that observed in wild-type slices (Fig. 6*A*). Similarly, the presence of statin in the recording electrode buffer

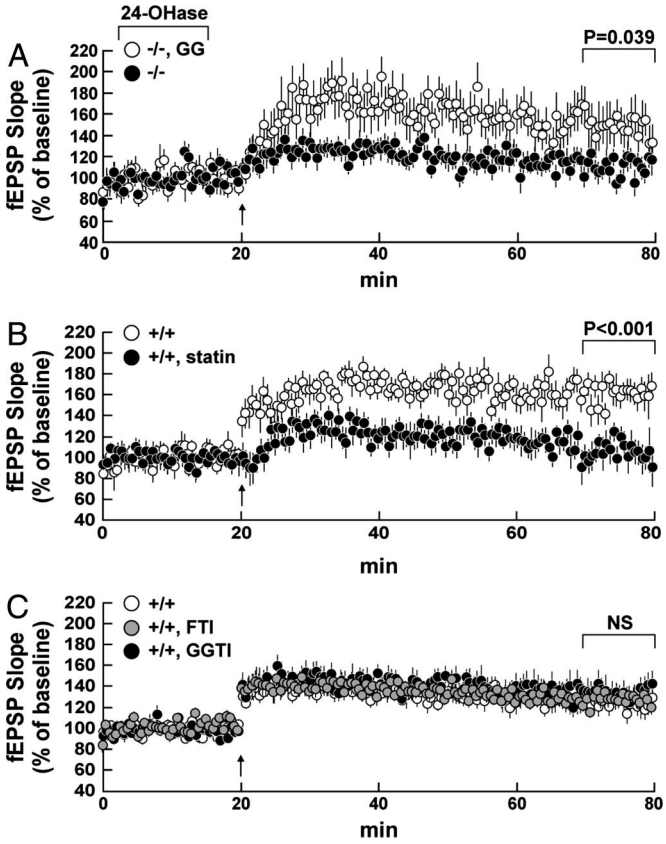

Fig. 6. Presence of geranylgeraniol, statin, or prenyltransferase inhibitors in recording electrode restores, inhibits, or has no effect on LTP, respectively. (*A*) LTP was measured in hippocampal slices from 24-hydroxylase knockout mice (−/−) using a recording electrode containing ACSF (●, $n = 22$) or ACSF supplemented with 0.2 mM geranylgeraniol (*GG*) (○, $n = 10$). LTP in the knockout slices was similar to that measured in wild-type slices. (*B*) LTP was measured in slices from wild-type mice (+/+) using a recording electrode containing ACSF (○, $n = 14$) or ACSF supplemented with 12.5 μM statin (●, $n = 11$). Delivery of the inhibitor via the recording electrode decreased LTP to levels observed in untreated knockout slices. (*C*) LTP was measured in wild-type slices using a recording electrode containing ACSF (○, $n = 12$), ACSF supplemented with 10 μM FTI- 277 (shaded circles, $n = 11$), or ACSF supplemented with 10 μM GGTI-2133 (●, $n = 12$). Neither prenyltransferase inhibitor affected potentiation.

inhibited LTP in wild-type slices, producing a response similar to that in knockout slices (Fig. 6*B*). Electrode administration of either of the prenyltransferase inhibitors to wild-type slices did not affect the induction or maintenance of LTP (Fig. 6*C*).

Discussion

The current findings provide a more complete picture of the role of geranylgeraniol in hippocampal LTP. The requirement for geranylgeraniol is chemically selective in that the related compound farnesol does not substitute, and is genetically selective for 24-hydroxylase deficiency in that hippocampal slices from a second animal model of LTP impairment, mice lacking apoE, do not respond to this polyisoprenol. Experiments with pharmacologic inhibitors and *gunmetal* mice indicate that geranylgeraniol action appears to be independent of acute protein isoprenylation. Characterization of 24-hydroxylase heterozygotes and time course experiments show that geranylgeraniol acts within 5 min and is required for both the induction and maintenance of LTP. Localized delivery of geranylgeraniol to the stratum radiatum, the dendritic trees emanating from CA1 hippocampal neurons, restored LTP in 24-hydroxylase knockout slices and conversely, delivery of a statin to these processes inhibited LTP in wild-type slices. We conclude that geranylgeraniol acts specifically, quickly, and locally to modulate LTP.

The mevalonate pathway gives rise to multiple isoprenoid end-products, including farnesyl diphosphate, geranylgeranyl diphosphate, cholesterol, and others (17). Several experiments reported here and elsewhere (9) indicate that of these end-products only geranylgeraniol will reverse the LTP defect in statin-treated wild-type slices or in those from 24-hydroxylase deficient mice. Although addition of exogenous geranylgeraniol restores LTP, we do not know whether the active compound *in situ* is the alcohol, the diphosphate, or another chemical form. Nor is it clear whether geranylgeraniol is active outside the neuron or must first be taken up by cells, perhaps metabolized, and then released for activity. The observation reported in Fig. S1 that farnesol is active in knockout slices has two implications along these lines. First, there must be a farnesol uptake mechanism in the hippocampus analogous to the polyisoprenol salvage pathway described in cultured cells (18) that allows neuronal enzymes to convert farnesol into geranylgeraniol via diphosphate intermediates. Second, if geranylgeraniol acts extracellularly to enhance synaptic strength, then a release mechanism must exist.

When the link between cholesterol turnover, geranylgeraniol synthesis, and LTP was initially made (9), we postulated that the isoprenoid requirement for long-term synaptic plasticity most likely reflected the need to posttranslationally modify a rapidly turning-over protein such as a member of the Rab, Rac, or Rho families, which are known to be important in this and other processes in the central nervous system (19). This postulate was tested here but no evidence to support a role for acute protein isoprenylation was obtained. To these ends, cell-permeable inhibitors of protein farnesyltransferase and geranylgeranyltransferases I and II when applied at the start and maintained throughout an LTP experiment, had no effects on potentiation in wild-type hippocampal slices (Fig. S2) or in slices from a hypomorph of geranylgeranyltransferase II, so-called *gunmetal* mice (Fig. 2). If slices were incubated with the inhibitors for long periods of time, a negative effect was realized (Fig. S3), indicating that these compounds were able to inhibit LTP but not when applied during the time frame in which geranylgeraniol is active. It must be emphasized that these interpretations are based on the assumptions that the three inhibitors used here gained immediate access to their enzyme targets and that a 70% reduction in geranylgeranyltransferase II activity functionally decreased isoprenylation in hippocampal slices from *gunmetal* mice.

Several conclusions are supported by the observations that mice heterozygous for the 24-hydroxylase mutation exhibit a different hippocampal LTP phenotype and an intermediate response in Morris water maze tests. First, the relationship between 24-hydroxylase activity and flux through the cholesterol biosynthetic pathway is linear, which is in contrast to most enzymes in metabolic pathways for which the relationship is non-linear (20). Second, mutation of the 24-hydroxylase gene causes a codominant phenotype, which is again rare in metabolomic genetics. These interpretations suggest that levels of geranylgeraniol are carefully titrated in the brain and that just enough of the isoprenoid is made to ensure normal synaptic plasticity. This conclusion is further supported by the observation that addition of geranylgeraniol to wild-type slices does not stimulate potentiation over and above that measured in untreated slices (9).

A third conclusion arising from characterization of 24-hydroxylase heterozygotes is that geranylgeraniol is required at two different times to establish LTP. Induction of LTP in heterozygous slices is normal at the beginning of an experiment but begins to decline at approximately 45 min, reaching

the baseline observed in homozygous slices by 60 min (Fig. 3*A*). LTP is maintained in heterozygous slices if geranylgeraniol is present throughout the experiment (Fig. 3*B*) or if a bolus of geranylgeraniol is added to the ACSF just before potentiation begins to decrease (between min 35 and 40, Fig. 4*A*). This response implies that there is enough geranylgeraniol in heterozygous slices to induce LTP but not enough to maintain potentiation over the duration of the recording period. Experiments in homozygous slices confirm the biphasic requirement for geranylgeraniol. Here, delivery of the polyisoprenol for a 5 min period encompassing the high frequency stimulation event produces an LTP response resembling that of heterozygous slices (Fig. 5*A*), and a double dose delivered at this time and between min 35–40 produces a wild-type LTP response (Fig. 5*B*). Similar but not identical temporal treatments of wild-type slices with statin support the existence of a biphasic response (Fig. 4 *C* and *D*).

By what mechanism does geranylgeraniol act to facilitate LTP at hippocampal CA1 synapses? The rapidity of the response and the narrow time windows in which the polyisoprenol acts point to an acute mechanism of action, which based on current knowledge could include an effect on glutamate receptor transport or activity, intracellular signaling, ion transport, glutamate metabolism, or other receptors (21). The chemical selectivity of the response together with the results of the electrode delivery experiments suggest that geranylgeraniol may be acting as a ligand for a cell surface receptor on the postsynaptic membrane. If this is the case, then geranylgeraniol may operate in an analogous manner but produce an opposite response to anandamide and 2-arachidonylglycerol, lipids which are released from postsynaptic neurons and act on the presynaptic membrane via the CB1 cannabinoid receptor to attenuate potentiation (22). Similarly, geranylgeraniol may act through one or more G protein-coupled receptors to influence LTP (23, 24). Future studies will be necessary to test this lipid signaling hypothesis and to determine the biochemical basis of the biphasic geranylgeraniol response.

Materials and Methods

Animals. Cholesterol 24-hydroxylase (gene symbol *Cyp46a1*) knockout mice (3) were maintained on a mixed strain background (C57BL/6J;129S6/SvEv) by matings between animals heterozygous for the introduced mutation. Wild-type littermates served as control animals in neurophysiological studies, whereas in behavioral studies non-littermate mice of similar hybrid background were used as controls. Mice heterozygous for the *gunmetal* allele (C57BL/6J-*Rabggta*^{gm}/J) and those homozygous for an induced apolipoprotein E (ApoE) mutation (B6.129P2-*Apoe*^{tm1Unc}/J) were purchased from the Jackson Laboratory. The University of Texas Southwestern Institutional Animal Care and Research Advisory committee approved all experimental procedures.

Electrophysiology. LTP measurements in hippocampal slices were carried out as described in ref. 9. A detailed description of these experiments is found in *SI Materials and Methods*.

Behavioral Studies. Morris water maze tests to determine spatial learning ability were performed with 3- to 4-month-old male mice as described in ref. 9. Data were collected in 4 separate experiments using a total of 40 wild-type mice, and 20 heterozygous and 30 homozygous 24-hydroxylase knockout mice. Results from the wild-type and homozygous mutant animals were reported previously (9) and are reproduced here for comparison purposes. A detailed description of the behavioral experiments is found in *SI Materials and Methods*.

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