Synaptic Transmission and Hippocampal Long-Term Potentiation in Olfactory Cyclic Nucleotide-Gated Channel Type 1 Null Mouse

ANGÈLE PARENT,¹ KAREN SCHRADER,² STEVEN D. MUNGER,² RANDALL R. REED,¹,² DAVID J. LINDEN,¹ AND GABRIELE V. RONNETT¹,³

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Parent, Angèle, Karen Schrader, Steven D. Munger, Randall R. Reed, David J. Linden, and Gabriele V. Ronnett. Synaptic transmission and hippocampal long-term potentiation in olfactory cyclic nucleotide-gated channel type 1 null mouse. J. Neurophysiol. 79: 3295–3301, 1998. Field potential recording was used to investigate properties of synaptic transmission and long-term potentiation (LTP) at Schaffer collateral-CA1 synapses in both hippocampal slices of mutant mice in which the α-subunit of the olfactory cyclic nucleotide-gated channel (α3/OCNC1) was rendered null and also in slices prepared from our wild-type (Wt) littermates. Several measures of basal synaptic transmission were unaltered in the OCNC1 knockout (KO), including maximum field excitatory postsynaptic potential (fEPSP) slope, maximum fEPSP and fiber volley amplitude, and the function relating fiber volley amplitude to fEPSP slope and paired-pulse facilitation. When a high-frequency stimulation protocol was used to induce LTP, similar responses were seen in both groups (KO: 1 min, 299 ± 50% (mean ± SE), 60 min, 123 ± 10%; Wt: 1 min, 287 ± 63%; 60 min, 132 ± 19%). However, on theta-burst stimulation, the initial amplitude of LTP was smaller (1 min after induction, 147 ± 16% of baseline) and the response decayed faster in the OCNC1 KO (60 min, 127 ± 18%) than in Wt (1 min, 200 ± 14%; 60 min, 169 ± 19%). Analysis of waveforms evoked by LTP-inducing tetanic stimuli revealed a similar difference between groups. The development of potentiation throughout the tetanic stimulus was similar in OCNC1 KO and Wt mice when high-frequency stimulation was used, but OCNC1 KO mice showed a significant decrease when compared with Wt mice receiving theta-burst stimulation. These results suggest that activation of cyclic nucleotide-gated channels may contribute to the induction of LTP by weaker, more physiological stimuli, possibly via Ca²⁺ influx.

INTRODUCTION

Cyclic nucleotide-gated (CNG) channels have a central role in sensory transduction in both vertebrate photoreceptors and olfactory receptor neurons (for review see Zagotta and Siegelbaum 1996). Several subunits have been identified in both neuronal and nonneuronal tissues and are classified as either α or β subunits. Vertebrate CNGs include those found in rod and cone photoreceptors, pinealocytes, olfactory neurons [olfactory cyclic nucleotide-gated channel (OCNC1) and OCNC2], and β subunits. Native olfactory CNG channels are likely to be heteromultimers that contain OCNC1 subunits, competent to form an ion channel as a homomultimer, and OCNC2 subunits, which modulate the heteromer by conferring an increased sensitivity to cyclic nucleotide (Bradley et al. 1994; Liman and Buck 1994). On binding of cyclic nucleotide to a domain in the cytoplasmic region, CNG channels rapidly open a nonselective cation pore. Although the olfactory CNG channel is sensitive to physiological intracellular concentrations of both adenosine 3′,5′-cyclic monophospho-cre (cAMP) and cyclic guanosine monophosphate (cGMP), the photoreceptor CNG channel is only sensitive to cGMP. In addition, the olfactory CNG channel carries a higher fractional Ca²⁺ current than the photoreceptor channel and is blocked by extracellular Mg²⁺ in a weakly voltage-dependent manner (Frings et al. 1995).

Recently it was shown that olfactory CNG channels have a distribution that is not limited to cells involved in sensory transduction, but are present in the brain as well (Bradley et al. 1997; El-Husseini et al. 1995; Kingston et al. 1996). Within the brain, OCNC1 mRNA has been detected by using reverse transcription—polymerase chain reaction (RT–PCR) in olfactory bulb, pituitary gland, cerebellum, neocortex, and hippocampus. In the hippocampus, in situ hybridization revealed OCNC1 mRNA in pyramidal cells of regions CA1–3 and granule cells of the dentate gyrus (Bradley et al. 1997; Kingston et al. 1996). Immunocytochemistry performed with a specific antiserum directed against a fusion protein corresponding to the C-terminal portion of rat OCNC1 showed immunoreactivity in both the cell body and dendritic layers of areas CA1–3 as well as in processes and growth cones of embryonic hippocampal neurons grown in culture (Bradley et al. 1997).

Electrophysiological evidence suggests that functional CNG channels are present in hippocampal neurons. Leinders-Zufall et al. (1995) made whole cell recordings from cultured embryonic rat hippocampal neurons and demonstrated that external application of 8Br-cGMP could activate an inward current that was blocked by Cd²⁺ and was highly permeable to Ca²⁺. Although this finding was consistent with the notion that cGMP directly gated this conductance, the possibility that the current was activated indirectly (e.g., by a protein kinase) could not be excluded. A more definitive analysis was performed by Bradley et al. (1997) who used excised inside-out patches from embryonic rat hippocampal neurons to show that low micromolar concentrations of cAMP and cGMP could activate an outwardly rectifying, flickery, Ni-insensitive conductance similar to that seen in olfactory receptor neurons. Thus it appears likely that functional CNG channels that include OCNC1 subunits are present in the synaptic layers of the hippocampus and that, once activated by cyclic nucleotide, these channels produce a
Ca\(^{2+}\) influx, a necessary trigger for several forms of synaptic plasticity in the hippocampus.

Cyclic nucleotide signaling was suggested to be central to both the short-term (Chavez-Noriega and Stevens 1994; Trudeau et al. 1996) and long-term (Frey et al. 1993; Huang et al. 1994; Weisskopf et al. 1994; Wu et al. 1995; Zhuo et al. 1994) modulation of hippocampal synaptic strength. Typically, increases in the concentration of cyclic nucleotide have been thought to exert their effects on synaptic strength through the activation of cyclic nucleotide dependent protein kinases. The present study is designed to test the hypothesis that activation of “olfactory” CNG channels may contribute to these processes as well.

**METHODS**

Transgenic mice

Genomic DNA encoding the OCNC1 cyclic nucleotide binding domain and several upstream exons was isolated from an Sau3A partial digest 129SV/J library in lambda-FixII (Stratagene). A Hind III/Not I fragment, beginning in the 3′ untranslated region (UTR) of the cDNA sequence and extending 4 kb into genomic sequences, was introduced downstream of the pgk-neo selection cassette. The 5′ flanking sequences for the construction of the gene disruption vector consisted of a 4.4 kb Hind III/Hind III fragment containing sequences above base pair (bp) 1416 (in rat OCNC1). Homologous recombination between the plasmid construct and the genomic OCNC1 gene would result in the loss of nucleotides 1416–2164 (for rat OCNC1) encoding the cyclic nucleotide binding domain and a portion of 3′ untranslated region sequence.

The homologous recombination vector was introduced into mouse 129 ES cells by electroporation and isolation of G-418 resistant colonies. Previous mapping studies had revealed that the mouse OCNC1 gene is located on the X chromosome. Homologous integrants were identified on the basis of hybridization with probes from the deleted regions as well as flanking sequence probes. Cells from a colony displaying homologous integration of the disruption vector were introduced into blastocyst stage C57Bl6 embryos. Highly chimeric animals were mated with C57BL6 females and the resulting offspring scored for germline transmission. Heterozygous that compared the KO and Wt groups. Two-way analysis of variation treatments included a 10-min treatment with proteinase K (not shown).

Bouin's, followed by overnight immersion at 4°C (Margolis 1985). The message for OCNC1 cannot be detected in olfactory receptor neurons and is absent from other cells in the epithelium.

**RESULTS**

To confirm the absence of a complete OCNC1 mRNA in situ hybridization for OCNC1 and OMP. The olfactory epithelium displays the highest expression of OCNC1 mRNA in the Wt mouse and therefore serves as the best test case for assessing deletion. OMP serves as a marker for olfactory receptor neurons; it is expressed in all olfactory receptor neurons and is absent from other cells in the epithelium (Margolis 1985). The message for OCNC1 cannot be detected in olfactory epithelium of KO mice (Fig. 1A), whereas it is readily visualized in Wt mice (Fig. 1B). In contrast, the OMP message was detected in both KO (Fig. 1C) and Wt (Fig. 1D) animals, demonstrating that olfactory receptor neurons were still present in the KO. In addition, polymerase chain reaction was performed to confirm that no detectable message remained for OCNC1 in the KO (data not shown).
The index of basal synaptic strength was unaltered, as was the tetrode stimulation amplitude, high levels of OCNC1 mRNA expression in neonatal neurons were induced during high-frequency stimulation (100 Hz, 200-ms interburst interval, 5 sets at 0.1 Hz). After a 60-min monitoring period in which both S1 and S2 test inputs were continued for another 60 min. When TBS stimulation was applied to S2 at t = 60 min, no difference in either PTP, measured 5 min after stimulation (Wt: 190 ± 34; KO: 189 ± 35; P = 0.978) or LTP measured 60 min after stimulation (Wt: 132 ± 19; KO: 123 ± 10; P = 0.711), was apparent.

To evaluate the effect of the OCNC1 KO on the earliest portions of the potentiated response, the waveform evoked by LTP-inducing tetanic stimulus (either theta-burst or high-frequency) was analyzed. The fEPSP slope of the individual synaptic responses comprising the tetanic stimulation was measured at various times during tetanic stimulation to give an index of the early development of the change in synaptic strength (Fig. 3, B and C). When high-frequency stimulation was applied, a steady increase in the fEPSP slope could be detected throughout the three 100 Hz × 1 s trains, reaching values of 323 ± 55 and 242 ± 55% (Wt and KO, respectively) for the first pulse in the last train. At no point during high-frequency stimulation could a significant difference in the fEPSP slope be detected among groups. In contrast, application of TBS produced a significant difference in the fEPSP slope at the first pulse of the third theta-burst set (Wt: 258 ± 20%; KO: 175 ± 21%; P < 0.0001). This parameter was also significantly larger in Wt compared with KO slices during the fourth and fifth theta-burst sets.

**DISCUSSION**

In OCNC1 KO mice the amplitude of LTP induced by theta-burst stimulation was significantly attenuated. A simple explanation for this finding is that during LTP induction, production of cyclic nucleotides (possibly via Ca$^{2+}$-sensitive adenylyl cyclase) activates OCNC1 channels and thus provides the Ca$^{2+}$ influx necessary to trigger LTP. Alternatively, high levels of OCNC1 mRNA expression in neonatal mouse hippocampus (S. Blackshaw, personal communication) may have long-lasting effects on synapse formation in the hippocampus. Although OCNC1 protein was detected (Ronnett, unpublished data)), OCNC1 mRNA was not detected in adult mouse hippocampus (Munger and Reed, unpublished data) in contrast to previous findings with the adult rat (Bradley et al. 1997; Kingston et al. 1996). Although the present report found no alteration in the basal properties of Schaffer collateral-CA1 synaptic transmission, it should be noted that the potentiation of LTP in the KO group (t = 60 min: Wt: 169 ± 19; KO: 127 ± 18; P = 0.04). When high-frequency stimulation was applied to S2 at t = 60 min, no difference in either PTP, measured 5 min after stimulation (Wt: 190 ± 34; KO: 189 ± 35; P = 0.978) or LTP measured 60 min after stimulation (Wt: 132 ± 19; KO: 123 ± 10; P = 0.711), was apparent.

**TABLE 1. Analysis of some parameters of basal synaptic transmission and short-term plasticity**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wt [n = 13 slices (7 mice)]</th>
<th>KO [n = 14 slices (7 mice)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum fiber volley amplitude, mV</td>
<td>-1.6 ± 0.2</td>
<td>-1.3 ± 0.1</td>
</tr>
<tr>
<td>Maximum fEPSP amplitude, mV</td>
<td>-5.1 ± 0.4</td>
<td>-5.2 ± 0.3</td>
</tr>
<tr>
<td>Maximum fEPSP slope, mV/ms</td>
<td>-3.4 ± 0.5</td>
<td>-3.1 ± 0.5</td>
</tr>
<tr>
<td>Paired-pulse facilitation, %</td>
<td>196 ± 15</td>
<td>179 ± 11</td>
</tr>
</tbody>
</table>

All values are expressed as a mean ± SE. Wt, wild-type; KO, knockout; fEPSP, field excitatory postsynaptic potential.
FIG. 2. Long-term potentiation (LTP) induced by high-frequency and theta-burst stimulation in OCNC1 KO mice. A: theta-burst stimulation (†) applied to the Schaffer collaterals of hippocampal area CA1 area through the stimulating electrode S1, induced input-specific LTP in slices from Wt (○) and OCNC1 KO mice (●). The amplitude of the potentiated response was significantly smaller in KO mice; * P < 0.0001, 2-way analysis of variance (ANOVA). B: stimulation of the 2nd pathway (S2) 1 h after LTP induction in the 1st pathway by using high-frequency stimulation (F) induced similar potentiation in Wt and KO mice (P = 0.538, 2-way ANOVA). Representative responses recorded at times t = 1 min, t = 1 min, and t = 60 min accompany each figure panel.

cautioned that there were several aspects of basal transmission that could not be addressed with field potential recording. These include voltage-dependence, kinetics, and ionic permeability of excitatory postsynaptic currents as well as the relative contribution of excitatory amino acid receptor subtypes. Hippocampal synapses are known to undergo a developmental conversion from higher to lower probability of release, facilitating the induction of long-term depression early in development and LTP at later points (Bolshakov and Siegelbaum 1995). It is possible that the absence of a functional OCNC1 protein disrupts or retards this developmental progression. In addition to synaptic transmission, it is not known whether the OCNC1 KO affects intrinsic conductances and/or second messenger systems of hippocampal neurons, including those that have been implicated in LTP induction or maintenance.

Both paired-pulse facilitation and PTP are short-term forms of synaptic plasticity that are thought to reflect temporary accumulation of free Ca$^{2+}$ in the presynaptic terminal (for review see Zucker 1989). In our experiments paired-pulse facilitation, which operates on the timescale of tens of milliseconds was unaltered, but PTP, which operates on the timescale of tens of seconds, was significantly reduced with theta-burst stimulation in the OCNC1 KO. This might reflect a slow and/or summating contribution of OCNC1 channels to presynaptic Ca$^{2+}$ flux. This effect could result in a greater degree of synaptic activation during tetanic stimulation and lead to a larger amplitude of LTP, as observed at later time points.

The induction requirements for LTP in the brain vary at different synapses. All models share an increase in Ca$^{2+}$ concentration as an initial step, either in the presynaptic compartment, as in mossy fiber-CA3 LTP, or in the postsynaptic compartment, as in Schaffer collateral/commissural-CA1 LTP. This Ca$^{2+}$ influx has the potential to activate a great number of second messenger systems including Ca$^{2+}$/CaM-sensitive adenyllyl cyclases. cAMP production was implicated in the induction of the early phase of mossy fiber LTP through activation of type 1 adenyllyl cyclase by Ca$^{2+}$/CaM via presynaptic voltage-gated Ca$^{2+}$ channels (Weis-skopf et al. 1994; Wu et al. 1994; cf. Johnston et al. 1992). The same model also was proposed for LTP of the parallel fiber-Purkinje neuron synapse in the cerebellum (Salin et al. 1996). In both of these cases, an LTP-like potentiation could be induced by application of the adenylate cyclase activator forskolin and tetanically induced LTP could be blocked by application of nucleotide analogs that interfere with cAMP-dependent protein kinase inhibitor (e.g., Rp-cAMP-S).
However, these compounds have similar effects on cyclic nucleotide binding sites in both CNG channels and cAMP-dependent protein kinases (Kramer and Tibbs 1996), confounding efforts to distinguish between these pathways.

cAMP signaling also was implicated in the late phase of LTP. At the Schaffer collateral/commissural-CA1 synapse, Sp-cAMP-S produced a slowly developing LTP-like effect that was dependent on protein synthesis, whereas Rp-cAMP-S attenuated the late phase of LTP produced by repeated high-frequency stimulation (Frey et al. 1993). The latter effect could also be produced by another inhibitor of cAMP-dependent protein kinase, KT5720 (Matthies and Reymann 1993). A late phase of mossy fiber-CA3 LTP produced by repeated tetanization also appears to require cAMP elevation (Huang et al. 1994). cAMP production may also be stimulated by a number of extrinsic modulatory projections to the hippocampus including those acting through β-adrenergic receptors (Hopkins and Johnston 1984; Huang and Kandel 1996; Thomas et al. 1996) and D1/D5 dopamine receptors (Huang and Kandel 1995; Otmakhova and Lisman 1996), founding efforts to distinguish between these pathways.

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![FIG. 3. Analysis of the synaptic input/output (I/O) relation and the tetanic stimulation waveforms. A: I/O relation between the fiber volley amplitude and the field excitatory postsynaptic potential (fEPSP) slope was determined over a range of stimulus intensities. Each point represents the mean of all slices tested (Wt; n = 13; KO; n = 14) for a narrow range of stimulus intensities. Error bars illustrate SE for both axes. No significant difference was found between Wt (○; fit with regression line $y = 2.04x/0.45, R^2 = 0.968$) and KO mice (●; fit with regression line $y = 2.21x/0.22, R^2 = 0.992$). B: analysis of theta-burst stimulation waveforms reveal a smaller increase of fEPSP slope during the LTP-inducing stimulation in the KO mice (●) compared with Wt mice (○). Analysis of fEPSP slopes of the 1st and 15th bursts of each set are shown. The roman numerals indicate successive sets; fEPSP slopes of the 1st pulse of the 3rd, 4th, and 5th sets were significantly larger in Wt compared with KO; * $P < 0.0001$. Traces on right: indicated burst (1st or 15th) with the corresponding bursts from the I and III sets overlayed. C: analysis of high-frequency stimulation tetanic waveforms demonstrates no difference between Wt and KO groups. fEPSP slopes of the 1st and the last (100th) pulse of each train are shown. The roman numerals indicate successive trains. Representative traces show the initial portions of the I and III trains overlayed.](image-url)
increased Ca$^{2+}$ influx could lead to a decrease in cAMP or cGMP. Because the activation profiles of cyclases and phosphodiesterases are not identical, they could work together to select for specific ranges of Ca$^{2+}$ concentrations that would favor the production of cyclic nucleotides.

Moreover, it has been suggested that LTP induction requires the generation of nitric oxide (NO) by postsynaptic Ca$^{2+}$/CaM-sensitive NO synthase and the subsequent retrograde diffusion of NO to the presynaptic terminal (for review see Schuman and Madison 1994). One potential presynaptic effector mechanism, soluble guanylyl cyclase, and the subsequent activation of a cGMP/cGMP-dependent protein kinase cascade (Zhuo et al. 1994) are supported by observations that presynaptic application of cGMP analogs promotes LTP induction whereas inhibitors of cGMP-dependent protein kinase can block LTP induction in pairs of cultured hippocampal neurons (Arancio et al. 1995). CNG channels in cone terminals can be activated by a retrograde NO/cGMP cascade, resulting in increased transmitter release onto horizontal cells (Savchenko et al. 1997). Direct gating of CNG channels by NO has been reported in retinal ganglion cells (Ahmad et al. 1994) and for ectopically expressed OCNC2 (Broillet and Firestein 1996), suggesting the possibility that similar mechanisms might be operative in hippocampal neurons.

In summary, LTP and PTP induced by theta-burst stimulation are attenuated in OCNC1 null mice, and this attenuation is not attributable to an alteration in basal synaptic strength or paired-pulse facilitation. These studies do not allow us to distinguish between long-lasting effects resulting from high-level expression of OCNC1 during neonatal development and direct effects of OCNC1 on LTP induction. In the second scenario, activation of OCNC1 contributes to LTP induction after TBS by contributing to Ca$^{2+}$ influx. When stronger stimulation (100 Hz) is used, the Ca$^{2+}$ flux produced by other routes is so large that removing the contribution of OCNC1 channels does not reduce the Ca$^{2+}$ level below the LTP induction threshold. Regardless of the mode of action, OCNC1 plays a significant role in long-term potentiation in the hippocampus, particularly that produced by weaker, more physiological stimuli.

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