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. RONNETT 1,3

1Department of Neuroscience, 2Howard Hughes Medical Institute, and 3Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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 (OCNC)1 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, nonselective cation pore. Although the olfactory CNG channel is sensitive to physiological intracellular concentrations of both adenosine 3′,5′-cyclic monophosphate (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 transcriptase–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 antibody 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. Lendners-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-sensitive 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; Weissskopf 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 were isolated from an Sau3A partial digest 129SV/J library in lambda-FixII (Stratagene). A Hind III/Not I fragment, beginning in the 3'terminated 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 complete cyclic nucleotide binding domain and a portion of 3'terminated region sequence.

The homologous recombination vector was introduced into mouse 129 ES cells by electroporation and isolation of G418 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 (10 not shown).

Bouin's, followed by overnight immersion at 4°C for 72 h, 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).

Electrophysiology

Mice (2- to 4-mo old) from five different litters were decapitated after a brief halothane anesthesia. The hippocampus was quickly dissected and prepared for conventional electrophysiological recordings. Transverse slices (500-μm thick) were cut by using a Vibratome in oxygenated (95% O2, 5% CO2) ice-cold, Ca2+-free artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 5 KCl, 2 MgSO4, 26 NaHCO3, 1.25 NaH2PO4, and 10 dextrose. The slices were held at room temperature for ≥60 min in an interface-type chamber (containing standard oxygenated ACSF containing 2 mM CaCl2) before transfer to an interface-type recording chamber perfused with oxygenated Ca2+-containing ACSF (1 ml/min), where the CA3 area was removed. The chamber was maintained at 33°C and all experiments were performed after allowing the tissue to equilibrate for ≥60 min in the interface chamber. Baseline measures of field excitatory postsynaptic potentials (fEPSP) were recorded for ≥30 min and were elicited at 0.017 Hz by alternately delivering a 0.02-ms constant-current pulse through one of two platinum concentric bipolar stimulation electrodes (FHC, Brunswick, ME) placed in the stratum radiatum of the CA1 region, on opposite sides of the recording site to stimulate Schaffer collateral/comissural fibers. The resulting extracellular potentials were monitored by using low-resistance glass pipettes (1–5 MΩ), filled with 1 M NaCl, placed in s. radiatum of CA1 to measure the fEPSP. In each experiment the maximal signal amplitude was first determined by gradually increasing the stimulus intensity until the signal amplitude reached a saturating level. The stimulus intensity was then decreased to evoke a test response that was ~30% of the maximal signal amplitude (32 ± 3% and 33 ± 3%, Wt and KO mice, respectively); typically this resulted in stimulation intensities of 0.05–0.15 mA. Paired-pulse facilitation was measured by using a 50-ms interpulse interval. Values of fEPSP slope represent the means ± SE of change expressed as a percentage of the value at t = 0 min. The basal synaptic transmission data were statistically evaluated by using a Student’s t test that compared the KO and Wt groups. Two-way analysis of variance was performed to compare statistical differences between both groups following posttetanic conditions over time. All compounds for electrophysiology were obtained from Sigma.
The index of basal synaptic strength was unaltered, as was a tively, high levels of OCNC1 mRNA expression in neonatal pulses were presented, high-frequency stimulation (100 Hz responses from both inputs (S1 and S2), one (S1) received published data) in contrast to previous findings with the adult (Ronnett, unpublished data), OCNC1 mRNA was not de-facilitation. tion) may have long-lasting effects on synapse formation in regression line was fitted to the relation between the fiber volley tive adenylyl cyclase) activates OCNC1 channels and thus... in the KO animal (A), but expression is normal in its Wt littermate (B). OMP expression is observed in both the KO (C) and Wt (D) animals, indicating the presence of olfactory receptor neurons in the olfactory epithelium of both animals. Decreased OMP labeling in the KO olfactory epithelium may reflect smaller numbers of olfactory receptor neurons or a diminished fitness of these cells.

Evaluation of some basal properties of synaptic transmission at the Schaffer collateral-CA1 synapse revealed no significant differences between OCNC1 KO and Wt mice (Table 1). Neither the maximum fEPSP slope or amplitude nor the fiber volley amplitude were altered. Furthermore, a regression line was fit to the relation between the fiber volley amplitude and the fEPSP slope (Table 1, Fig. 3A). This index of basal synaptic strength was unaltered, as was a measure of very short-term synaptic plasticity, paired-pulse facilitation.

To evaluate long-term potentiation (LTP), two stimulating electrodes were used to activate nonoverlapping populations of fibers in the s. radiatum. After acquisition of baseline responses from both inputs (S1 and S2), one (S1) received theta-burst stimulation (TBS; 15 bursts of 4 pulses at 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 pulses were presented, high-frequency stimulation (100 Hz × 1 s trains) was delivered to S2 and monitoring of both inputs was continued for another 60 min. When TBS was applied to S1, input specific LTP was induced in both groups (Fig. 2). However, a significant difference between KO and Wt was apparent within 5 min of TBS (Wt: 247 ± 18%; KO: 187 ± 20%; P = 0.004). At this point the potentiated response is dominated by posttetanic potentiation (PTP), an independent phenomenon that persists even when LTP induction is blocked with postsynaptic manipulations [e.g., Ca²⁺ chelator loading or N-methyl-d-aspartate (NMDA) receptor antagonists]. However, whereas early responses to tetanic stimulation are dominated by PTP, a pure measure of PTP would require tetanization in the presence of an NMDA receptor antagonist, a manipulation that we did not perform. At later time points, significant attenuation of LTP is also apparent 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.

To evaluate the effect of the OCNC1 KO on the earliest portions of the potentiated response, the waveform evoked by the 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 fEPSP slope could be detected throughout the three 100 Hz × 1 s trains, reaching values of 232 ± 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 fEPSP slope be detected among groups. In contrast, application of TBS produced a significant difference in 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 Ca²⁺-sensitive adenylyl cyclase) activates OCNC1 channels and thus provides the Ca²⁺ 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

**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</td>
<td>−1.6 ± 0.2</td>
<td>−1.3 ± 0.1</td>
</tr>
<tr>
<td>amplitude, mV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum fEPSP amplitude</td>
<td>−5.1 ± 0.4</td>
<td>−5.2 ± 0.3</td>
</tr>
<tr>
<td>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>
<tr>
<td>%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as a mean ± SE. Wt, wild-type; KO, knockout; fEPSP, field excitatory postsynaptic potential.
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 summing 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 adenylyl cyclases. cAMP production was implicated in the induction of the early phase of mossy fiber LTP through activation of type 1 adenylyl cyclase by Ca\(^{2+}\)/CaM via presynaptic voltage-gated Ca\(^{2+}\) channels (Weisskopf 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).
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 revealed 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.

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 1996; Otmakhova and Lisman 1996), and cAMP signaling also was implicated in the late phase of the activation of which also has been suggested to facilitate LTP induction and/or maintenance.

Although forskolin and Sp-cAMP-S produce robust potentiation at the mossy fiber-CA3 synapse, they produce very weak or no potentiation (Blitzer et al. 1995; Thomas et al. 1996; Weisskopf et al. 1994) at the Schaffer collateral/commissural-CA1 synapse. The present observation that basal synaptic strength is not altered in the Schaffer collateral/commissural-CA1 synapses of OCNC1 KO mice is consistent with these reports.

Because hippocampal neurons express Ca$^{2+}$/CaM-sensitive phosphodiesterase, it is possible that the CNG-depen
dent 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.

We thank L. Kramer for manuscript preparation. This work was supported by the Develbiss Fund (D. J. Linden and G. V. Ronnett), the McKnight Foundation (D. J. Linden and G. V. Ronnett), the National Alliance for Research on Schizophrenia and Depression (D. J. Linden), a fellowship from the Fonds de la Recherche en Santé du Quebec (A. Parent), National Institute of Deafness and Other Communications Disorders Grant RO1-DC-02979 to G. V. Ronnett, and the Howard Hughes Medical Institute (R. R. Reed).

Address for reprint requests: G. V. Ronnett, Dept. of Neurology, Johns Hopkins University School of Medicine, 725 North Wolfe St., Baltimore, MD 21205.

Received 19 December 1997; accepted in final form 3 March 1998.

REFERENCES


Thomas, M. J., Moody, T. D., Makinson, M., and O’Dell, T. J. Activ...


