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Enhanced LTP of primary afferent neurotransmission in AMPA receptor GluR2-deficient mice

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Abstract

Ca^{2+}-permeable-AMPA receptors (AMPARs) are expressed in the superficial dorsal horn (SDH, laminae I/II) of the spinal cord, the area involved in transmission and modulation of sensory information, including nociception. A possible role of Ca^{2+}-permeable-AMPARs in synaptic strengthening has been suggested in postnatal DH cultures, but their role in the long-lasting activity-dependent synaptic plasticity of primary afferent neurotransmission in the adult mouse SDH has not been investigated. In the present study the role of Ca^{2+}-permeable-AMPARs in the regulation of long-lasting synaptic plasticity, specifically long-term potentiation (LTP) and long-term depression (LTD) in the SDH, was investigated using mice deficient in AMPAR GluR2 subunit. We show here that the GluR2 mutants exhibited no changes in passive membrane properties, but a significant increase in rectification of excitatory postsynaptic currents, the finding suggesting increased expression of Ca^{2+}-permeable-AMPARs. In the absence of GluR2, high-frequency stimulation (HFS) of small-diameter primary afferent fibers induced LTP that is enhanced and non-saturating in the SDH at both primary afferent Aδ- and/or C-fibers monosynaptic and polysynaptic pathways, whereas neuronal excitability and paired-pulse depression were normal. The LTP could be induced in the presence of the NMDA receptor antagonist D-AP5, and L-type Ca^{2+} channel blockers, suggesting that Ca^{2+}-permeable-AMPARs are sufficient to induce LTP in the SDH neurons of adult mouse spinal cord. In contrast, the induction of HFS-LTD is reduced in the SDH of GluR2 mutants. These results suggest an important role for AMPAR GluR2 subunit in regulating synaptic plasticity with potential relevance for long-lasting hypersensitivity in pathological states.

Keywords: Ca^{2+}-permeable-AMPA receptor; Spinal cord; LTP; GluR2 knockout mice

1. Introduction

Superficial dorsal horn (SDH, laminae I/II), including substantia gelatinosa (SG, lamina II) of spinal cord, is the preferential site of termination of small-diameter primary afferent fibers that respond to noxious stimuli (Kumazawa and Perl, 1978; Light and Perl, 1979a,b; Sugiura et al., 1986, 1989; Gerber and Randic, 1989a,b; Yoshimura and Jessell, 1990). SDH neurons include projection neurons, that conduct excitatory signals to higher brain for further processing, as well as local inhibitory and excitatory interneurons regulating output of projection neurons (Coggeshall and Willis, 1991).

Glutamate receptors (GluRs) of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) type

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(AMPARs), composed of heteromeric assemblies of GluR1-4 subunits, mediate fast excitatory synaptic transmission in mammalian central nervous system (CNS) including spinal cord. Of the four subunits, only GluR2 has a positively-charged amino acid arginine (R) at position 586 of transmembrane segment 2, instead of neutral glutamine (Q) found in other subunits. This position called Q/R site is subjected to RNA editing (Sommer et al., 1991), and is the main site controlling Ca^{2+} permeation of recombinant AMPARs (Burnashev et al., 1992). The presence of edited GluR2 hinders Ca^{2+} permeation through AMPAR, and confers linear current–voltage (*I–V*) relationship (Burnashev et al., 1992). In contrast, AMPARs assembled without edited GluR2 are Ca^{2+}-permeable, and show inwardly rectifying *I–V* relationship. Since GluR2 is fully edited throughout development, and widely expressed in adult rodent CNS (Petralia et al., 1997), only a subset of neurons, predominantly GABAergic interneurons, expresses Ca^{2+}-permeable-AMPARs (Geiger et al., 1995). In spinal cord, GluR2 is highly expressed in SG (Tölle et al., 1993; Lu et al., 2002), presumably indicating prominent existence of AMPARs that are not Ca^{2+}-permeable. However, Engelman et al., 1999 suggested that NK1 receptor-positive lamina I projection neurons use Ca^{2+}-permeable-AMPARs to mediate or modulate synaptic strength, as reported for cultured DH neurons (Gu et al., 1996). In addition, there is evidence that GABAergic neurons in lamina II express low density of GluR2, but large levels of GluR1, and have Ca^{2+}-permeable-AMPARs (Todd and Sullivan, 1990; Spike et al., 1998; Albuquerque et al., 1999; Engelman et al., 1999, 2006). These findings are in agreement with expression of Ca^{2+}-permeable-AMPARs in inhibitory interneurons in other CNS regions (Geiger et al., 1995). On the other hand, a recent study demonstrated that increased expression of Ca^{2+}-permeable-AMPARs, by disrupting GluR2 gene, facilitates behavioral responses to mechanical/thermal stimuli following inflammation (Hartmann et al., 2004). Functional significance of GluR2 in synaptic plasticity has been best studied for long-term potentiation (LTP) in hippocampus and the regulation of synaptic strengthening underlying activity-dependent learning (Jia et al., 1996; Takahashi et al., 2003). Considering evidence that molecular mechanisms of learning and memory are also operational in pain pathways (Sandkühler, 2000), we have tested if GluR2 plays role(s) in induction and/or expression of LTP and long-term depression (LTD) of primary afferent neurotransmission in adult mouse SDH (Youn et al., 2000). In the present study, we compared effects of high-frequency stimulation (HFS) of dorsal roots on Aδ- and/or C-fibers-evoked monosynaptic and polysynaptic excitatory postsynaptic potentials (EPSPs) in SDH neurons from wild-type (+/+) and GluR2 subunit-lacking mice.

2. Materials and methods

2.1. Slice preparation

Transverse lumbar (L4–5) spinal cord slices (400–500 μm) with 8–15 mm long dorsal roots were prepared under isoflurane anesthesia from 63- to 121-day-old +/+, (129SvEv/C57BL6) and GluR2 knockout ($^{-/-}$; 129SvEv/C57BL6) mice of both sexes (Sans et al., 2003). Slices were cut in oxygenated (95% O_2, 5% CO_2) Krebs-bicarbonate solution (4 °C) containing (mM): 124 NaCl, 5 KCl, 1.2 KH_2PO_4, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 26 $NaHCO_3$, 10 glucose; pH 7.4, on a vibratome. After slices were incubated for >1 h in holding chamber to recover, a slice was superfused (3 ml/min) in recording chamber with oxygenated recording solution (33 ± 1 °C; same composition to the slicing solution, except for 128 mM NaCl and 1.9 mM KCl).

2.2. Intracellular recordings

Under visual control, a single fiberglass (o.d. and i.d., 1.00 and 0.58 mm, respectively; AM Systems, Everett, WA, USA) microelectrode (4 M potassium acetate, pH 7.2; 140–220 MΩ) was placed in the lamina I and SG region, recognized as translucent band in lamina II of spinal DH. Neurons were impaled by oscillating capacity compensation circuit of Axoclamp 2A (Axon Instruments, Union city, CA, USA). Digidata 1200 and pClamp (version 6 and 8) were used for data acquisition and analysis. Most recordings were obtained from neurons with a stable resting membrane potential (V_m; >−60 mV) and with overshooting action potentials. Synaptic potentials were evoked by single shocks of electrical stimulation (0.01–0.5 ms, 5–35 V; repeated at 2 min interval) of primary afferent fibers with a bipolar platinum electrode. Identification of EPSPs was based on the criteria as previously described (Youn and Randic, 2004). Briefly, EPSPs with a calculated conduction velocity (root length/latency) of 1.5–15 m/s were classified as Aδ, and those with <1.5 m/s as C-fibers. Identification of 'monosynaptic' Aδ-fiber-mediated EPSPs was based on constant latencies and absence of failures with repetitive stimulation at 10 Hz; 'polysynaptic' in the case of variable latencies or existence of failures. After a stable baseline recording (at least 10 min), HFS (100 Hz for 1 s, delivered 3 times at 0.1 Hz) was applied to induce either LTP or LTD (Randic et al., 1993). Similar protocol was used in other regions of the brain (Artola et al., 1990; Bröcher et al., 1992; Laezza and Dingledine, 2004). The induction of LTP or LTD was defined as at least a 15% increase or decrease in peak amplitude of EPSPs, respectively, that was maintained for a minimum of 20 min following HFS. Each change in EPSP amplitude was expressed as percentage of baseline control at 20 min following HFS. Statistical significance of data has been assessed relative to baseline control responses and between genotypes (unpaired Student's *t*-test; significance, $P < 0.05$). Probability assigned to different effects in individual genotypes was statistically compared by Fisher's exact test. Data are expressed as means ± SEM. In most of experiments, each neuron was recorded in a different slice.

2.3. Whole-cell patch clamp recordings

For *I–V* relationships of dorsal root-evoked excitatory postsynaptic currents (EPSCs), whole-cell patch clamp recordings were made with Axopatch 200B (Axon Instruments) under blind condition. Borosilicate glass patch pipettes (6–12 MΩ) were filled with internal solution (mM: 140 $CsMeSO_4$, 10 Na–HEPES, 10 EGTA, 2 NaCl, 1 $CaCl_2$, 2 Tris–ATP, 0.3 Tris–GTP, 5 QX314, pH 7.2, 295–300 mOsm). EPSCs were evoked by electrical stimulation of dorsal roots (0.1–0.5 ms pulses of 3–30 V at 0.033 Hz) using bipolar platinum wire electrode, filtered at 2 kHz, sampled at 10 kHz, and analyzed with pCLAMP (version 9). Series resistance was measured directly from the amplifier and compensated by 70%. The recording was terminated if series resistance (about 8–20 MΩ) changed by more than 20%. For *I–V* relationships, AMPAR-mediated EPSCs were evoked at each holding potential ranging from −60 to +40 mV (20-mV increments), and the peak amplitude of EPSCs was measured to plot against each potential. To quantify the degree of rectification of EPSCs, we calculated rectification index (RI) of EPSCs, defined as ratio of the peak EPSC amplitude at +40 mV divided by the predicted value at +40 mV extrapolated from linear fitting of EPSCs at negative holding potentials (Liu and Cull-Candy, 2000).

2.4. Chemicals

Bicuculline methiodide (BMI), strychnine, dizocilpine maleate (MK801) and philanthotoxin-343 (PhTx) were purchased from Sigma–Aldrich (St. Louis, MO, USA); D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), (*S*)−α-methyl-4-carboxyphenylglycine (MCPG), nifedipine and nimodipine from Tocris Cookson (Ellisville, MO, USA).

3. Results

3.1. Passive membrane properties and presynaptic mechanisms in wild-type and GluR2 mutant mice

Conventional intracellular and whole-cell patch clamp recordings from SDH neurons in transverse lumbar spinal slices revealed no significant differences between +/+ and $GluR2^{-/-}$ mice in resting V_m (intracellular: -72.2 ± 1.3 mV ($n = 39$) *vs.* -73.4 ± 1.4 ($n = 31$); whole-cell: -61.8 ± 2.7 mV ($n = 8$) *vs.* -59.9 ± 1.7 ($n = 7$)), membrane input resistance (intracellular: 212.9 ± 25.1 MΩ ($n = 23$) *vs.* 215.6 ± 31.2 ($n = 27$); whole-cell: 204.2 ± 29.3 MΩ ($n = 10$) *vs.* 230.9 ± 49.3 ($n = 11$)), threshold to evoke Aδ-fiber-evoked monosynaptic EPSP (4.7 ± 0.3 V ($n = 30$) at 0.1 ms *vs.* 5.4 ± 0.5 ($n = 20$)), and conduction velocity for primary afferent fiber-evoked EPSPs (Aδ-fiber: 4.4 ± 0.6 m/s ($n = 16$) *vs.* 3.5 ± 0.4 ($n = 15$); C-fiber: 0.6 ± 0.1 m/s ($n = 7$) *vs.* 0.6 ± 0.2 ($n = 4$)), respectively. We did not observe any significant difference in the extent of paired-pulse depression of EPSPs, calculated by the ratio of 2nd EPSP to 1st EPSP amplitude (an interpulse interval of 100 ms), between slices obtained from +/+ and $GluR2^{-/-}$ mice (0.62 ± 0.10, $n = 6$ neurons, and 0.62 ± 0.06, $n = 8$ neurons, respectively), the data suggesting that in $GluR2^{-/-}$ mice, the excitability of primary afferent fibers and neurotransmitter release is likely to be normal.

3.2. Whole-cell recordings of evoked EPSCs in SDH neurons

To examine the effect of genetic deletion of GluR2 subunit on synaptic membrane properties, we first established *I–V* relationships of AMPAR-mediated EPSCs in +/+ and $GluR2^{-/-}$ slices in the presence of 10–20 μM BMI, 2 μM strychnine and 50–100 μM D-AP5, antagonists for $GABA_A$, glycine and *N*-methyl-D-aspartate receptors (NMDARs), respectively. To quantify the degree of rectification, we calculated RI of EPSCs. As shown in Fig. 1a and b, $GluR2^{-/-}$ mice showed much stronger inward rectifications in *I–V* relationship than +/+ mice. The RI of $GluR2^{-/-}$ mice (0.07 ± 0.03, $n = 6$ neurons/4 mice, $P < 0.01$) was significantly decreased when compared with that of +/+ mice (0.41 ± 0.08, 6 neurons/5 mice). Because the *I–V* relationship and the RI are closely correlated with the Ca^{2+}-permeability (Isa et al., 1996) and the subunit composition of AMPAR channels (Liu and Cull-Candy, 2000; Sans et al., 2003), these results indicate that the Ca^{2+}-permeability through AMPARs expressed on the postsynaptic membrane of SDH neurons may efficiently be increased due to the genetic deletion of GluR2 subunit. The existence of Ca^{2+}-permeable-AMPARs in $GluR2^{-/-}$ mice was further supported by statistically significant depression of EPSC amplitude ($52.2 \pm 5.9\%$ of control, $n = 4$; Fig. 1c) by a small dose of PhTx (1 μM), an open channel blocker for Ca^{2+}-permeable non-NMDAR.

3.3. Synaptic plasticity: Enhanced LTP of evoked EPSPs from Aδ- and/or C-fibers, but reduced probability of LTD induction in $GluR2^{-/-}$ mice

To examine the role of GluR2 in the regulation of synaptic plasticity of primary afferent fibers-activated synaptic transmission in the SDH, we compared two forms of long-lasting synaptic changes (LTP/LTD) induced by HFS of dorsal roots (Randic et al., 1993) between +/+ and $GluR2^{-/-}$ mice (Fig. 1d and e). The magnitude of LTP induced by HFS of dorsal roots was significantly enhanced in $GluR2^{-/-}$ mice ($123.3 \pm 9.5\%$, $n = 12$ (out of 24; 50%) neurons tested, 10 slices, 9 +/+ mice *vs.* $177.0 \pm 15.7\%$, $n = 17$ (out of

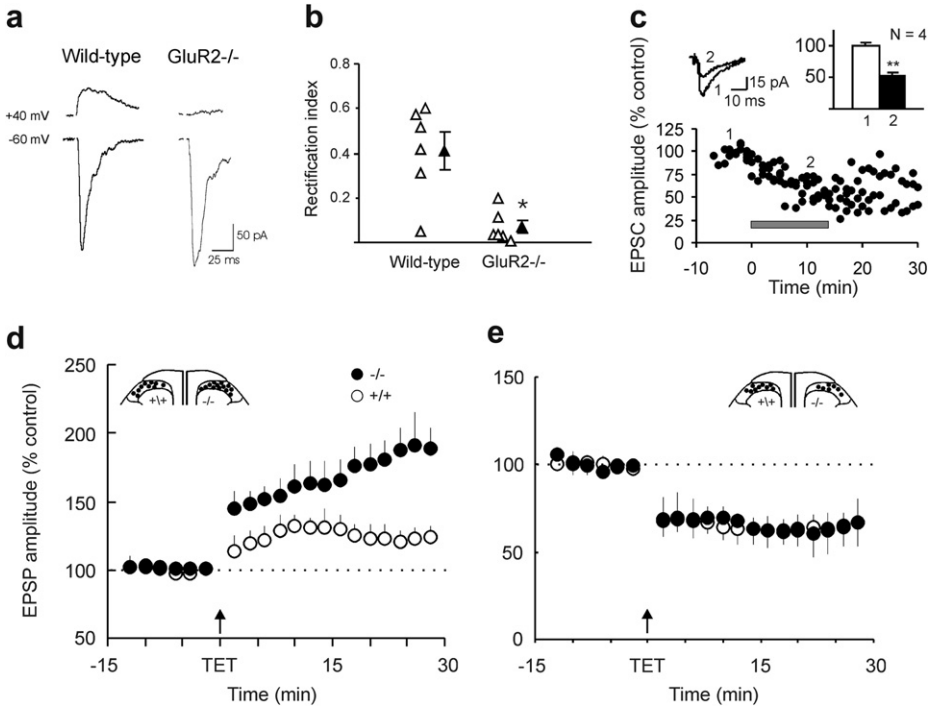

Fig. 1. Rectification properties of synaptic currents and synaptic plasticity of excitatory transmission in SDH neurons. Traces (a) are examples of primary afferents-evoked EPSCs from +/+ and GluR2$^{-/-}$ SDH neurons at +40 and –60 mV in the presence of 50 μM D-AP5, 10 μM BMI and 2 μM strychnine. A scatter diagram (b) represents each rectification index from six +/+ and six GluR2$^{-/-}$ SDH neurons. *Filled triangles* (in b) indicate mean rectification index ± SEM for each group ($^{*}P < 0.05$). Bath application of PhTx (c; 1 μM, 14 min; *gray bar*) depressed EPSC amplitude, to 52.2 ± 5.9% of control ($n = 4$, *inset histogram* in c). Summary graphs demonstrate the magnitude and the time course of LTP (d) and LTD (e) induced by the same protocol of HFS (TET, *arrows*) in +/+ and GluR2$^{-/-}$ mice. *Insets* show the approximate location of all tested SDH cells.

24; 71%) neurons tested, 13 slices, 12 GluR2$^{-/-}$ mice, $P < 0.01$). However, LTD induced by the identical stimulation protocol was indistinguishable in the magnitude between +/+ and GluR2$^{-/-}$ mice (62.9 ± 9.8%, $n = 11$ (out of 24; 46%) neurons, 11 slices, 11 +/+ mice *vs.* 62.4 ± 9.1%, $n = 7$ (out of 24; 29%) neurons, 7 slices, 7 GluR2$^{-/-}$ mice, $P > 0.05$). In contrast, we observed that the low-frequency dorsal root stimulation (900 pulses at 1 Hz) induced LTP in a knockout cell (140.9%), whereas LTD (74.6%) was recorded in two +/+ neurons. This interesting finding was not further pursued.

Although the enhancement of LTP of primary afferent neurotransmission has been shown in the SDH region of AMPAR GluR2$^{-/-}$ mice (Fig. 1d), it is not known whether this effect can be induced in primary afferent Aδ- and C-fiber monosynaptic and polysynaptic pathways. Therefore we compared the magnitude of LTP of evoked EPSPs from Aδ- and/or C-fibers in slices obtained from +/+ and GluR2$^{-/-}$ mice. The LTP of EPSPs in GluR2 mutants was significantly enhanced in the SDH cells receiving monosynaptic (162.9 ± 11.2% of control, $n = 5$, $P < 0.01$ *vs.* +/+; Fig. 2a and d) and polysynaptic (176.4 ± 21.22%, $n = 6$, $P < 0.05$ *vs.* +/+; Fig. 2d) inputs from Aδ-, and C-fibers (234.6 ± 40.5% of control, $n = 3$, $P < 0.05$ *vs.* +/+; Fig. 2b and d). Almost all of a monosynaptic Aδ-fiber-mediated EPSP during LTP was blocked by 10 μM CNQX, a non-NMDAR antagonist, indicating that LTP of AMPAR-mediated EPSPs is enhanced in GluR2 mutants.

As the induction of LTP at primary afferent synapses critically depended on the activation of NMDAR (Randic et al., 1993), we investigated whether the Ca^{2+}-permeable-AMPARs can substitute for NMDARs during LTP induction. As shown in Fig. 3c and d, in the presence of 50–100 μM D-AP5, GluR2$^{-/-}$ SDH neurons predominantly exhibited LTP (140.3 ± 8.6%, $n = 7$ (out of 11; 64%) neurons tested, 7 slices, 7 mice; $P < 0.05$) of monosynaptic Aδ-fiber ($n = 3$; Fig. 3a)- and C-fiber ($n = 4$; Fig. 3b)-evoked EPSPs; the rest of neurons receiving inputs from Aδ-fibers showed LTD ($n = 3$) or no change ($n = 1$). However, HFS predominantly produced LTD ($n = 7$ (out of 10; 70%) neurons tested, 7 slices, 7 mice; 64.2 ± 5.2%; $P < 0.05$) of Aδ-fibers (monosynaptic, $n = 2$; polysynaptic, $n = 2$)- and C-fibers ($n = 3$; Fig. 3b)-activated transmission in +/+ SDH neurons; the rest of SDH neurons receiving inputs from monosynaptic and/or polysynaptic Aδ-fibers showed LTP ($n = 1$) or no change ($n = 2$; Fig. 3a). Our results, showing the incomplete blockade of LTP induction in

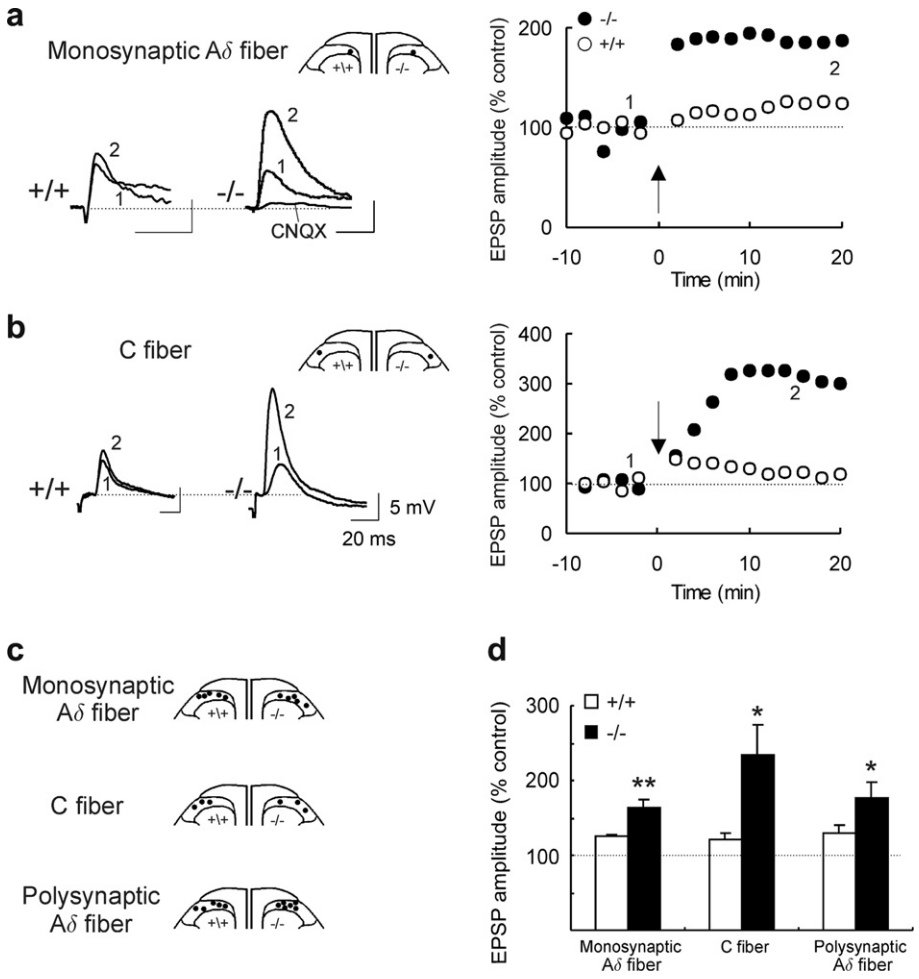

Fig. 2. Enhanced LTP in GluR2$^{-/-}$SDH neurons receiving different synaptic inputs. Superimposed traces (*left column*) and time-course graphs (*right column*) demonstrate the enhanced LTP of monosynaptic Aδ-fiber (a)- and C-fiber (b)-evoked EPSPs, induced by HFS (*arrows*). Numbers on the graphs indicate the corresponding time of the sampled traces. CNQX, a non-NMDAR antagonist, almost completely blocked the monosynaptic Aδ-fiber-evoked EPSP (a). *Insets* show the location of SDH neurons recorded. Histogram (d) compares the magnitude of LTP of EPSPs evoked from monosynaptic and polysynaptic Aδ-fiber, and C-fiber between +/+ and GluR2$^{-/-}$ SDH neurons (cell locations, in c) ($^{*}P < 0.05$; $^{**}P < 0.01$).

the GluR2$^{-/-}$ mice by the NMDAR antagonist, indicate that the LTP was no longer entirely NMDAR-dependent. It should be also noted that the probability of LTP induction was significantly increased, and that of LTD is significantly decreased, in GluR2 mutants (LTP, 64%; LTD, 27%; $P < 0.05$, Fisher's exact test), when compared with those in +/+ (LTP, 10%; LTD, 70%). On the other hand, the magnitude of LTD in the presence of MCPG, group I and II metabotropic GluR antagonist, and D-AP5 in +/+ slices ($64.4 \pm 8.2\%$, $n = 6$) was not significantly different from that in D-AP5 alone ($P > 0.05$), indicating that other mechanisms, such as cannabinoid receptors and dopamine receptors (Malenka and Bear, 2004; Anwyl, 2006), may be involved in the HFS-induced LTD in the mouse spinal cord SDH.

We next investigated whether the residual LTP in the presence of D-AP5 could be due to Ca^{2+} influx via the L-type high voltage activated Ca^{2+}(HVAC) channels. However, when we induced the LTP of monosynaptic Aδ-fibers-evoked EPSPs (Fig. 4a, left panel, lower traces) in GluR2$^{-/-}$ cells, in the presence of 100 μM D-AP5 and 20 μM nifedipine, the L-type HVAC channel antagonist, the mean peak EPSP amplitude at 20 min after tetanus was $123.8 \pm 4.1\%$ of control ($n = 3$; Fig. 4b), which was not significantly different from those recorded in D-AP5 alone ($P > 0.05$, Fig. 3d). The LTP was also induced in a GluR2$^{-/-}$ cell in the presence of D-AP5, MK-801 (10 μM), an open channel blocker of NMDAR, and nimodipine (10 μM), another HVAC antagonist (118% of control). In contrast only LTD ($79.3 \pm 6.2\%$ of control, $n = 4$; Fig. 4b) was recorded in the +/+ cells. Therefore these results suggest that GluR2$^{-/-}$ mice have about 140% of increase in synaptic efficacy in the absence of NMDAR and HVAC contribution.

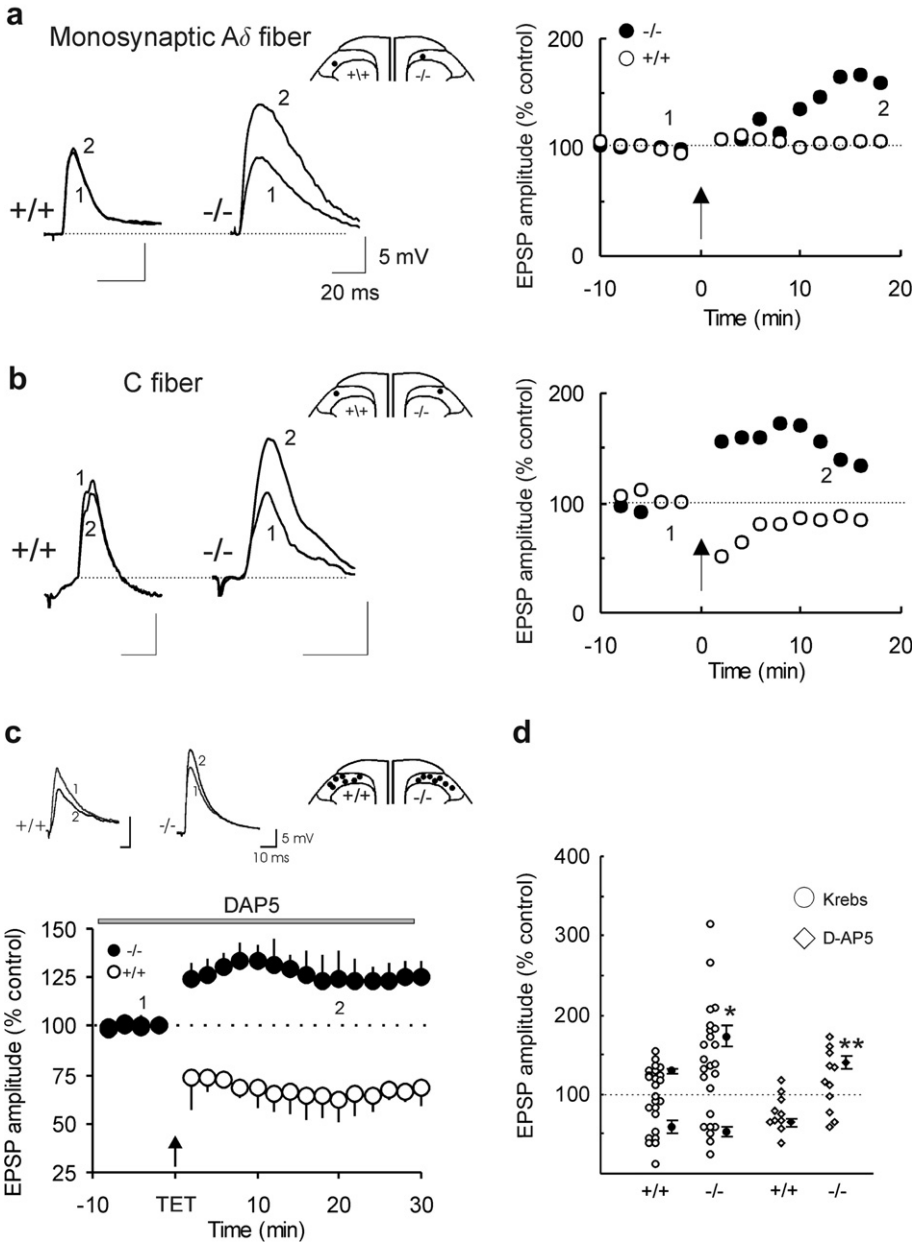

Fig. 3. LTP in the presence of NMDAR blockade in GluR2$^{-/-}$ mice. HFS (*arrows*) induced LTP of monosynaptic Aδ-fiber (a)- and C-fiber (b)-evoked EPSPs in GluR2$^{-/-}$ mice, but rather no change (a) or LTD (b) in +/+ mice, in the presence of 50–100 μM D-AP5. The summarized time-course graph (c) from all SDH neurons (cell locations, in *inset*) recorded in the presence of the NMDAR antagonist D-AP5 demonstrates LTP in GluR2$^{-/-}$ mice and LTD in +/+ mice following HFS (*arrow*). The superimposed traces displayed above the graph (c) are individual EPSPs recorded from SDH neurons in +/+ and GluR2$^{-/-}$ mice. Numbers on the graphs indicate the corresponding time of the sampled traces. Bar indicates the period of drug application. A scatter diagram (d) demonstrates changes of EPSP amplitudes in individual SDH neurons, represented as percent of baseline control at 18–20 min after HFS. Points with error bar in the diagram (d) indicate means ± SEM (% of baseline control) averaged in LTP (*above dotted line*) and LTD (*below dotted line*) groups from +/+ and GluR2$^{-/-}$ mice in Krebs solution (*filled circles*) and all SDH neurons from each genotype in D-AP5-containing solution (*filled diagonals*). ($^{*}P < 0.05$ or $^{**}P < 0.01$.)

4. Discussion

Activation of Ca^{2+}-permeable-AMPARs contributes to LTP in hippocampus (Jia et al., 1996; Plant et al., 2006) and amygdala (Mahanty and Sah, 1998). A possible role of Ca^{2+}-permeable-AMPARs in synaptic strengthening has been suggested, using early postnatal DH neuron cultures (Gu et al., 1996). However, their role in LTP of primary afferent transmission in adult SDH, including SG, has not been investigated. Therefore, we decided to test whether GluR2 plays any role in induction of LTP and/or LTD at primary afferents-SDH neuron synapses in GluR2$^{-/-}$ slices, since GluR2 mRNA transcripts are in particular highly expressed in

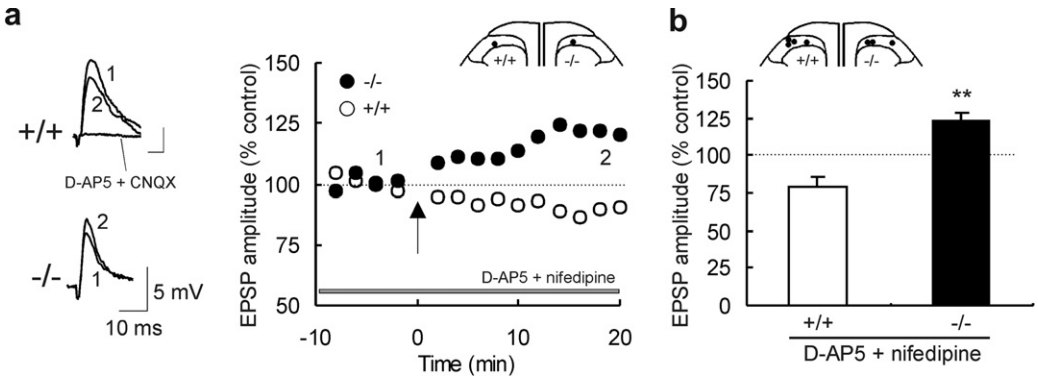

Fig. 4. LTP in the presence of L-type Ca^{2+} channel blockade in GluR2$^{-/-}$ mice. HFS-induced LTP in GluR2$^{-/-}$ mice, but small LTD in +/+ mice, of monosynaptic Aδ-fiber-evoked EPSPs in the presence of 100 μM D-AP5 and 20 μM nifedipine (a). The percent change of EPSP amplitude 18–20 min after HFS in the presence of D-AP5 and nifedipine was significantly different between +/+ and GluR2$^{-/-}$ mice (b).

SG (Tölle et al., 1993). Our novel findings are: (1) in the absence of GluR2, magnitude of HFS-induced LTP is enhanced in SDH at both Aδ- and/or C-fiber monosynaptic and polysynaptic pathways; (2) Ca^{2+} influx via L-type Ca^{2+} channels appears not to be critically involved in LTP induction in GluR2$^{-/-}$ mice; (3) probability of HFS-LTD induction is significantly reduced in GluR2$^{-/-}$ mice. These results suggest that GluR2 may play an important role in regulating LTP/LTD, and pain behavior in spinal SDH.

AMPARs containing Q/R-edited GluR2 have lower Ca^{2+} permeability and distinct gating properties *in vitro*, compared with receptor channels assembled without this subunit (Burnashev et al., 1992). In addition, loss of GluR2 in GluR2$^{-/-}$CA1 pyramidal neurons resulted in 9-fold increase in a relative Ca^{2+} permeability following kainate application when compared with that of +/+ mice (Jia et al., 1996). These results, together with observations made in hippocampal slices from GluR2-editing-deficient mice (Brusa et al., 1995), support a crucial role for GluR2 in inhibiting Ca^{2+} influx via AMPARs *in vitro*. The degree of Ca^{2+}-permeable-AMPARs expression in spinal DH is variable, depending on DH subregions, types of terminating fibers, or neuronal properties (Popratiloff et al., 1996; Engelman et al., 1999; Lu et al., 2002). In contrast, all AMPARs existing in spinal GluR2$^{-/-}$ slices are expected to be Ca^{2+}-permeable. This expectation was likely confirmed by our present result that all GluR2$^{-/-}$ SDH neurons showed strong inward rectification in *I–V* relationship of AMPAR-mediated EPSCs.

There is convincing evidence for significant role for postsynaptic NMDARs and Ca^{2+} in LTP induction in hippocampal CA1 (Malenka and Bear, 2004), as well as SDH (Randic et al., 1993). Since NMDAR activity is strongly modulated by Ca^{2+}-sensitive kinases activity (Randic, 1996), it is conceivable that in GluR2$^{-/-}$ mice Ca^{2+} influx associated with activation of Ca^{2+}-permeable-AMPARs could modify NMDAR function in SDH through protein phosphorylation (Malenka and Bear, 2004). However, this is unlikely since in the presence of D-AP5, HFS still produced LTP in GluR2$^{-/-}$ SDH neurons, the finding indicating that LTP induction is no longer entirely NMDAR-dependent. Moreover, whole-cell data obtained from CA1 hippocampal neurons did not show any change in NMDA component of synaptic current in GluR2 mutants, and low-frequency stimulation-induced LTD, another NMDAR-dependent phenomenon, was not changed (Jia et al., 1996). These findings support hypothesis that Ca^{2+} influx via GluR2-deficient AMPARs does not alter NMDA component of synaptic response and is sufficient to produce LTP. Since synaptic Ca^{2+} fluxes associated with Ca^{2+}-permeable-AMPAR activation will be present at negative V_m, while depolarized V_m will favor greater Ca^{2+} fluxes through NMDARs, it may be expected that Ca^{2+}-permeable-AMPARs affect synaptic strength with different sensitivity than NMDARs. It is therefore likely that Ca^{2+} influx through AMPARs at resting V_m (Burnashev et al., 1992) may synergistically participate in LTP induction, together with Ca^{2+} influx through NMDARs at depolarized potentials (Malenka and Bear, 2004).

Evidence suggests the presence of NMDAR-independent LTP in CA1 Schaeffer collateral synapse that can be blocked by L-type HVAC channel antagonist nifedipine (Grover and Teyler, 1990). Such a mechanism could underlie NMDAR-independent LTP in GluR2$^{-/-}$ mice. However, we found that LTP in GluR2$^{-/-}$ mice could also be preserved under blockade of HVAC channels by nifedipine, in the presence of D-AP5, or additional blockade of depolarization-activated channels to NMDARs by whole-cell voltage-clamping (−70 mV; data not shown) (Mahanty and Sah, 1998). These results show that HVAC channels do not play a major role in NMDAR-independent LTP, consistent with a role for Ca^{2+}-permeable-AMPARs in its induction.

Previous studies showed that in a subpopulation of SDH neurons the same HFS (100 Hz) can elicit either

LTP or LTD depending on the level of V_m of postsynaptic neuron during HFS (Randic et al., 1993), in agreement with the results obtained in the brain (Artola et al., 1990; Bröcher et al., 1992; Laezza and Dingledine, 2004; Malenka and Bear, 2004), while low-frequency (1 Hz) stimulation of Aδ-fibers induces only LTD in SDH (Sandkühler et al., 1997). Although cellular mechanism underlying HFS-induced LTD is still not well understood, several mechanisms such as a level of V_m (Randic et al., 1993), a rise in postsynaptic Ca^{2+} concentration (Cheng and Randic, 2003), and AMPAR endocytosis via phosphorylation of GluR2 on serine-880 (Chung et al., 2003) and interaction of GluR2 with PICK 1 (Steinberg et al., 2006) have been suggested. However, the result, normal AMPAR endocytosis and hippocampal LTD in GluR2/GluR3 deficient mice (Jia et al., 1996; Meng et al., 2003), suggests that GluR1 contains all the necessary molecular determinants for LTD induction. In this context, our finding showing decreased probability of HFS-LTD in the presence of D-AP5 in GluR2$^{-/-}$ mice may involve other mechanisms such as aberrant formation of GluR complexes (Sans et al., 2003) and a simple masking of LTD by high probability of LTP induction.

Synaptic plasticity is fundamental to many neurobiological functions including pain. Long-lasting activity-dependent changes in efficacy of glutamatergic synapses in pain pathways (central sensitization) contribute to pain following tissue or nerve injury (Randic, 1996; Gerber et al., 2000; Sandkühler, 2000; Woolf and Salter, 2000; Ji et al., 2003). Recent evidence suggests that AMPAR is an important element of long-lasting modifications in efficacy of central synapses analogous to LTP and LTD, and that these modifications are required for behavioral or cognitive plasticity generated by experience (Malenka and Bear, 2004). Furthermore, in spinal DH, postsynaptic Ca^{2+}-permeable-AMPARs may mediate nociceptive synaptic transmission onto NK1 receptor-carrying projection neurons (Tong and MacDermott, 2006) and onto GABAergic interneurons (Albuquerque et al., 1999). Besides postsynaptic localization, Engelman et al. (2006) recently provided the first demonstration of functional presynaptic Ca^{2+}-permeable-AMPARs on inhibitory interneurons in DH that regulate GABA and glycine release onto interneurons in lamina II and onto lamina I NK1 receptor-expressing neurons in DH. However, functional significance of Ca^{2+}-permeable-AMPARs in spinal processing of nociceptive inputs has been studied in the context of LTP of synaptic transmission in native spinal SDH neurons of GluR2$^{-/-}$ mice (Youn et al., 2000). In the present study, we demonstrated that in the absence of GluR2, HFS-induced LTP is enhanced at both primary afferent Aδ and/or C monosynaptic and polysynaptic pathways. The enhancement of synaptic activation of projection neurons is expected to increase nociceptive signaling, whereas a decrease or increase is predicted for GABAergic neurons depending on identity of targets of these inhibitory interneurons. In the latter context, it is of interest our finding that probability of HFS-LTD induction is significantly reduced in GluR2$^{-/-}$ mice in the presence of NMDAR blockade. The NMDAR-independent mechanisms for facilitating excitatory synaptic transmission are potentially important in pain system (Sorkin et al., 1999, 2001; Stanfa et al., 2000; Jones and Sorkin, 2004). In particular, in a subpopulation of SDH neurons, AMPARs lacking edited form of GluR2 are expressed, and they allow Ca^{2+} influx sufficient to produce long-lasting facilitation of synaptic transmission in SDH neurons (Youn et al., 2000). Because Ca^{2+}-permeable-AMPARs are expressed on projection neurons, and as well on GABAergic interneurons, their contribution to processing of nociceptive inputs *in vivo* has proven unpredictable. In this context the results of Hartmann et al. (2004) are very relevant, since they demonstrated that GluR1 and GluR2 reciprocally modulate spinal synaptic plasticity and inflammatory pain. They reported decrease in the number of Ca^{2+}-permeable-AMPARs and density of AMPA channel currents in spinal neurons of GluR1$^{-/-}$, that is accompanied by a loss of nociceptive plasticity *in vitro*, and a reduction in acute inflammatory hyperalgesia *in vivo*. In contrast, an increase in spinal Ca^{2+}-permeable-AMPARs in GluR2$^{-/-}$ mice facilitated nociceptive plasticity and enhanced long-lasting inflammatory hyperalgesia. Indeed, a decrease in hyperalgesia has been seen after administration of a Ca^{2+}-permeable non-NMDAR antagonist *in vivo* (Sorkin et al., 1999, 2001). The evidence provided by Hartmann et al. (2004), coupled with our data shown here, provides strong support for the emerging view that "central sensitization" shares some similarities with cellular and molecular mechanisms of hippocampal and spinal LTP (Randic et al., 1993; Randic, 1996; Willis, 1997; Sandkühler, 2000; Ji et al., 2003). In addition, data obtained in our study, and those of Hartmann et al. (2004), predict that lack of GluR2-containing AMPARs would lead to long-lasting nociceptive hypersensitivity in pathological states. Therefore, it would be of interest to investigate potential change in efficiency of GluR2 mRNA editing in pathological pain states following inflammation or nerve injury (Carlton et al., 1998; Zhou et al., 2001; Garry et al., 2003; Guan et al., 2003), for example, an impairment of GluR2 RNA editing in human patients suffering from amyotrophic lateral sclerosis (Kawahara et al., 2004).

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