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PICK1-mediated Glutamate Receptor Subunit 2 (GluR2) Trafficking Contributes to Cell Death in Oxygen/Glucose-deprived Hippocampal Neurons*

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Oxygen and glucose deprivation (OGD) induces delayed cell death in hippocampal CA1 neurons via Ca^{2+}/Zn^{2+}-permeable, GluR2-lacking AMPA receptors (AMPARs). Following OGD, synaptic AMPAR currents in hippocampal neurons show marked inward rectification and increased sensitivity to channel blockers selective for GluR2-lacking AMPARs. This occurs via two mechanisms: a delayed down-regulation of GluR2 mRNA expression and a rapid internalization of GluR2-containing AMPARs during the OGD insult, which are replaced by GluR2-lacking receptors. The mechanisms that underlie this rapid change in subunit composition are unknown. Here, we demonstrate that this trafficking event shares features in common with events that mediate long term depression and long term potentiation and is initiated by the activation of *N*-methyl-D-aspartic acid receptors. Using biochemical and electrophysiological approaches, we show that peptides that interfere with PICK1 PDZ domain interactions block the OGD-induced switch in subunit composition, implicating PICK1 in restricting GluR2 from synapses during OGD. Furthermore, we show that GluR2-lacking AMPARs that arise at synapses during OGD as a result of PICK1 PDZ interactions are involved in OGD-induced delayed cell death. This work demonstrates that PICK1 plays a crucial role in the response to OGD that results in altered synaptic transmission and neuronal death and has implications for our understanding of the molecular mechanisms that underlie cell death during stroke.

Oxygen and glucose deprivation (OGD)[3] associated with transient global ischemia induces delayed cell death, particularly in hippocampal CA1 pyramidal cells (1–3), a phenomenon that involves Ca^{2+}/Zn^{2+}-permeable, GluR2-lacking AMPARs (4). AMPARs are heteromeric complexes of subunits GluR1–4 (5), and most AMPARs in the hippocampus contain GluR2, which renders them calcium-impermeable and results in a marked inward rectification in their current-voltage relationship (6–8). Ischemia induces a delayed down-regulation of GluR2 mRNA and protein expression (4, 9–11), resulting in enhanced AMPAR-mediated Ca^{2+} and Zn^{2+} influx into CA1 neurons (10, 12). In these neurons, AMPAR-mediated postsynaptic currents (EPSCs) show marked inward rectification 1–2 days following ischemia and increased sensitivity to 1-naphthyl acetyl spermine (NASPM), a channel blocker selective for GluR2-lacking AMPARs (13–16). Blockade of these channels at 9–40 h following ischemia is neuroprotective, indicating a crucial role for Ca^{2+}-permeable AMPARs in ischemic cell death (16).

In addition to delayed changes in AMPAR subunit composition as a result of altered mRNA expression, it was recently reported that Ca^{2+}-permable, GluR2-lacking AMPARs are targeted to synaptic sites via membrane trafficking at much earlier times during OGD (17). This subunit rearrangement involves endocytosis of AMPARs containing GluR2 complexed with GluR1/3, followed by exocytosis of GluR2-lacking receptors containing GluR1/3 (17). However, the molecular mechanisms behind this trafficking event are unknown, and furthermore, it is not known whether these trafficking-mediated changes in AMPAR subunit composition contribute to delayed cell death.

AMPAR trafficking is a well studied phenomenon because of its crucial involvement in long term depression (LTD) and long term potentiation (LTP), activity-dependent forms of synaptic plasticity thought to underlie learning and memory. AMPAR endocytosis, exocytosis, and more recently subunit-switching events (brought about by trafficking that involves endo/exocytosis) are central to the necessary changes in synaptic receptor complement (7, 18–20). It is possible that similar mechanisms regulate AMPAR trafficking during OGD.

PICK1 is a PDZ and BAR (Bin-amphiphysin-Rus) domain-containing protein that binds, via the PDZ domain, to a number of membrane proteins including AMPAR subunits GluR2/3. This interaction is required for AMPAR internalization from the synaptic plasma membrane in response to Ca^{2+} influx via NMDAR activation in hippocampal neurons (21–23). This process is the major mechanism that underlies the reduction in synaptic strength in LTD. Furthermore, PICK1-mediated trafficking has recently emerged as a mechanism that regulates the GluR2 content of synaptic receptors, which in turn determines their Ca^{2+} permeability (7, 20). This is likely to be of profound importance in both plasticity and pathological mechanisms. Importantly, PICK1 overexpression has been shown to induce a shift in synaptic AMPAR subunit composition in hippocampal

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[3] The abbreviations used are: OGD, oxygen and glucose deprivation; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPAR, AMPA receptor; NMDA, *N*-methyl-D-aspartic acid; NMDAR, NMDA receptor; LTP, long term potentiation; LTD, long term depression; EPSC, excitatory postsynaptic current; NASPM, 1-naphthyl acetyl spermine; PI, propidium iodide; ABP, AMPAR-binding protein; GRIP, glutamate receptor-binding protein.

CA1 neurons, resulting in inwardly rectifying AMPAR EPSCs via reduced surface GluR2 and no change in GluR1 (24). This suggests that PICK1 may mediate the rapid switch in subunit composition occurring during OGD (17). Here, we demonstrate that the OGD-induced switch in AMPAR subunit composition is dependent on PICK1 PDZ interactions, and importantly, that this early trafficking event that occurs during OGD contributes to the signaling that results in delayed neuronal death.

EXPERIMENTAL PROCEDURES

Electrophysiology—Brain slices were prepared from postnatal day 13–15 male Wistar rats following a lethal dose of anesthetic (isoflurane inhalation). Brains were dissected in ice-cold aCSF (in mM, 119 NaCl, 2.5 KCl, 1 $NaH_2PO_4 \cdot H_2O$, 26.2 $NaHCO_3$, 10 glucose, 2.5 $CaCl_2$, 9 $MgSO_4$) saturated with 95% O_2 and 5% CO_2. Transverse hippocampal slices 300–400 μm thick were cut using a vibratome (DTK-1000, DSK, Japan), and slices were stored at room temperature for at least 1 h before use. Before being transferred to the submerged recording chamber, the connections between CA3 and CA1 were cut. Slices were placed in a submerged recording chamber perfused with aCSF (as above) at room temperature with the addition of 50 μM picrotoxin. CA1 pyramidal cells were visualized using infrared differential interference contrast optics on a Zeiss Axioskop microscope. Patch electrodes with a resistance of 4–5 megaohms were pulled from borosilicate filamented glass capillaries. Pipettes were filled with intracellular solution containing (in mM) 130 $CsMeSO_3$, 8 NaCl, 5 QX314-Cl, 10 HEPES, 0.5 EGTA, 4 Mg-ATP, 0.3 Na-GTP, 0.1 spermine, 0.1 bestatin, 0.1 leupeptin, 0.1 pepstatin and set to pH 7.2, 280–285 mosM. Peptides were added at a final concentration of 30 μM. Recordings from CA1 pyramidal neurons were made with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA), filtered at 5 kHz, and digitized at 10 kHz using a data acquisition board and Signal acquisition software (CED, Cambridge, UK). Cells were voltage-clamped at −70 mV. Series resistance was monitored throughout the experiments, and cells that showed >20% change or were greater than 25 megaohms were discarded from subsequent analysis. Synaptic responses were evoked with 100-μs square voltage steps applied at 0.1 Hz through a bipolar stimulating electrode (FHC) located in the stratum radiatum. D-AP5 and picrotoxin were purchased from Tocris Bioscience.

Rectification was calculated as the mean EPSC amplitude at +40 mV divided by the mean EPSC amplitude at −70 mV. Mean EPSCs were averages of at least 20 individual sweeps. Comparisons of EPSC rectification before and after OGD were assessed by a paired Student's *t* test with the null hypothesis rejected if $p < 0.05$. Data are plotted as the mean ± S.E.

Neuronal Cultures, Sindbis Virus Infection, and Plasmid Transfection—Primary neuronal cultures were prepared from embryonic day 18 Wistar rat hippocampi. Sindbis viruses were prepared according to the Sindbis Expression System manual (Invitrogen).[4] Infections were carried out as described (25). Transfections were carried out using Lipofectamine 2000.

[4] Invitrogen, *Sindbis Expression System Manual*, Invitrogen, Carlsbad, CA.

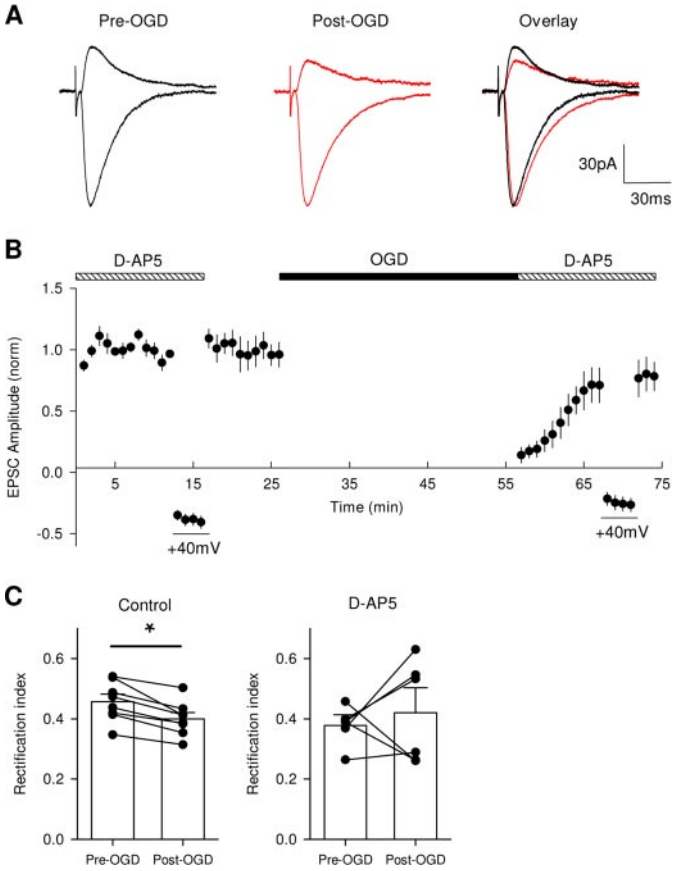

FIGURE 1. **OGD causes an NMDA receptor-dependent increase in AMPA receptor rectification.** *A*, OGD produces a reduction in the amplitude of AMPA receptor-mediated synaptic responses recorded at +40 mV in comparison with −70 mV. Example traces show synaptic responses recorded while holding the CA1 pyramidal cell at −70 mV and +40 mV pre-OGD (*black*) and post-OGD (*red*). Traces are shown *overlaid* on the *right*. *B*, rectification is measured by recording synaptic responses at −70 mV and +40 mV in D-AP5 before and after 30 min of OGD. D-AP5 is washed out of the slice before OGD is commenced. Average normalized synaptic response is shown for eight experiments. *C*, the rectification index decreases after OGD in control conditions corresponding to an increase in AMPAR rectification ($n = 8$). The rectification index change for each experiment is represented by the *filled circles*. The decrease is blocked by the presence of D-AP5 during OGD. *, $p < 0.05$.

Oxygen/Glucose Deprivation—For biotinylations and cell death assays, neuronal cultures were exposed to OGD by transferring them to culture medium containing sucrose instead of glucose, which had been saturated with 95% N_2, 5% CO_2. After three washes in this medium, cultures were placed in a prewarmed Billups-Rothenberg modular incubator chamber in a 37 °C incubator and flushed with 95% N_2, 5% CO_2 for 10 min. The air-tight chamber was then sealed, and following various time points, cultures were transferred back to normal conditioned medium (cell death assays) or processed for subsequent experimentation (biotinylations). For electrophysiology experiments, slices were perfused with 95% N_2, 5% CO_2-saturated aCSF containing sucrose in place of glucose for 30 min followed by perfusion with normal aCSF. Stimulation was ceased during the OGD period.

Surface Biotinylation—Following OGD, cultures at 18–21 days *in vitro* were chilled on ice, washed in ice-cold PBS, and incubated with 0.25 mg/ml Sulfo-NHS-SS-Biotin (Pierce) in PBS for 10 min on ice. After washing three times in PBS plus 1

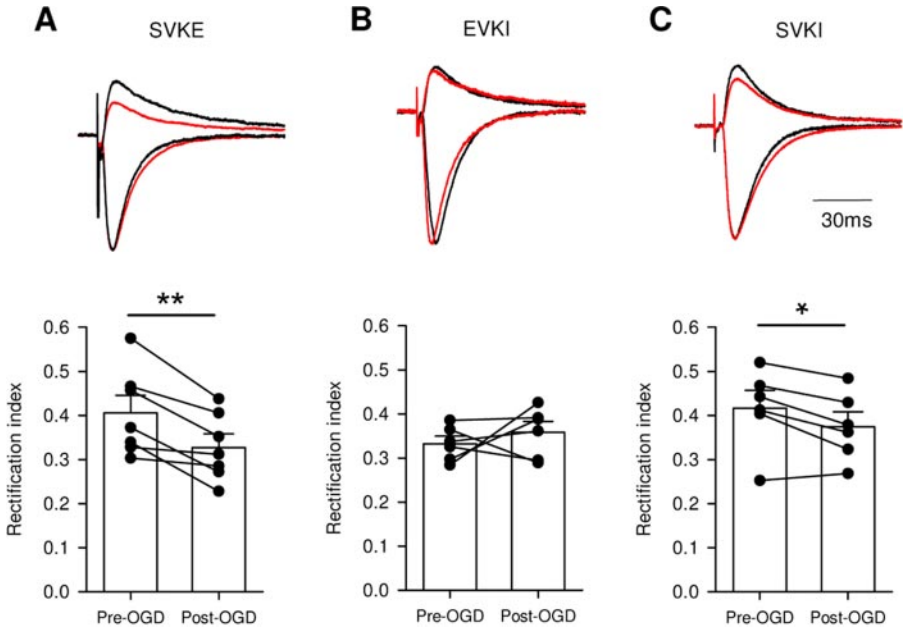

FIGURE 2. **OGD-induced increase in AMPA receptor rectification is blocked by inhibiting PICK1 PDZ domain interactions.** *Top panels*, example traces recorded at −70 mV and +40 mV pre-OGD (*black*) and post-OGD (*red*). Post-OGD traces are scaled so that −70 mV peaks are identical. *Bottom panels*, average change in rectification ($n = 7$) with individual experiments represented by *circles*. *A*, control peptide pep2-SVKE has no effect on the OGD-induced decrease in the rectification index. **, $p < 0.005$. *B*, PICK1-specific peptide pep2-EVKI completely blocks the OGD-induced change in the rectification index. *C*, PICK1 and ABP/GRIP-blocking peptide pep2-SVKI does not completely block OGD-induced change in the rectification index. *, $p < 0.05$.

mg/ml bovine serum albumin and three times in PBS, cells were lysed in 500 ml of lysis buffer (25 mM HEPES (pH 7.2), 150 mM NaCl, 1% Triton X-100, 0.2% SDS). After centrifugation, lysate was incubated with streptavidin-agarose beads for 3 h at 4 °C, washed four times in lysis buffer, and bound protein detected by Western blotting.

Quantification of Western Blots—Films of Western blots from at least four identical independent experiments were scanned and analyzed using Image J. A ratio of values for bands representing surface over those for total GluR2 was determined for a given condition. *Error bars* are standard errors, and paired *t* tests were carried out to determine significant differences.

Cell Death Assays—30 h following OGD, cultures at 20–22 days *in vitro* were stained with propidium iodide (PI; 4 μg/ml) and Hoechst stain (2 μg/ml) for 1 h before imaging. A field of view was defined as a scan across the width of the coverslip. Within this area, the proportion of Hoechst-positive nuclei that were PI-positive was counted. For each experiment, the average of four fields of view was calculated per condition. The experiment was then repeated four times (peptides) or seven times (NASPM). *Error bars* are standard errors, and paired *t* tests were carried out to determine significant differences.

RESULTS

OGD Induces an NMDAR-dependent Change in AMPAR Rectification—AMPAR EPSC rectification is a well established assay for the presence of GluR2-containing AMPARs. 99% of GluR2 subunits in CA1 pyramidal cells are edited at the Q/R site, preventing susceptibility to block by endogenous polyamines. AMPARs lacking GluR2 are blocked by polyamines in a voltage-dependent manner, with the degree of blockade much greater at positive membrane potentials (5–7). These properties result in a linear current-voltage relationship for GluR2-containing AMPARs but an inwardly rectifying relationship for those lacking GluR2. It is therefore possible to assess the proportion of GluR2-containing AMPARs at the synapse by measuring the inward rectification displayed by the current-voltage relationship.

We measured the rectification of AMPAR-EPSCs by making whole-cell voltage clamp recordings from CA1 pyramidal neurons in acute hippocampal slices and stimulating the Schaffer collaterals. Cells were voltage-clamped at −70 mV and +40 mV in the presence of the NMDAR antagonist D-AP5 (50 μM) to measure the degree of AMPAR rectification (Fig. 1, *A* and *B*). After D-AP5 washout, a 30-min OGD protocol was then applied to the slice (see "Experimental Procedures"), after which D-AP5 was reapplied and the EPSC amplitude slowly recovered over 10–15 min. Once the EPSC amplitude reached stability, rectification was again measured at −70 mV and +40 mV (Fig. 1*B*). The rectification index was measured by dividing the EPSC amplitude at +40 mV by that at −70 mV; therefore a decrease in the index corresponds to an increase in the degree of current-voltage rectification. 30 min of OGD produced a decrease in the rectification index in control experiments (pre-OGD = 0.46 ± 0.02, post-OGD = 0.40 ± 0.02, $n = 8$, $p < 0.001$). This effect was blocked if D-AP5 was present throughout the OGD protocol (pre-OGD = 0.38 ± 0.03, post-OGD = 0.42 ± 0.07, $n = 6$, $p = 0.6$) (Fig. 1*C*). Time-matched control experiments that were identical except without OGD produced no change in the rectification index (pre-OGD = 0.35 ± 0.03, post-OGD = 0.37 ± 0.05, $n = 6$, $p = 0.58$, data not shown).

To ensure that OGD did not induce a change in the EPSC reversal potential, we performed separate experiments recording responses at −70, −50, −30, and −10 mV before and after OGD. By fitting a straight line through the data points, we calculated the EPSC reversal potential and found no change after OGD (pre-OGD 0.9 ± 3 mV, post-OGD 3.2 ± 5 mV, $n = 3$, $p = 0.49$, data not shown).

The OGD-induced Change in AMPAR-EPSC Rectification Is PICK1-dependent—The increase in rectification following OGD suggests a rapid decrease in the proportion of synaptic GluR2-containing AMPARs relative to GluR2-lacking AMPARs during the OGD insult. Because PDZ domain interactions with GluR2 are known to regulate AMPAR trafficking (19, 26, 27), we carried out experiments to determine the role of such interactions during OGD using peptides corresponding to the C terminus of GluR2 to block interactions with the PDZ

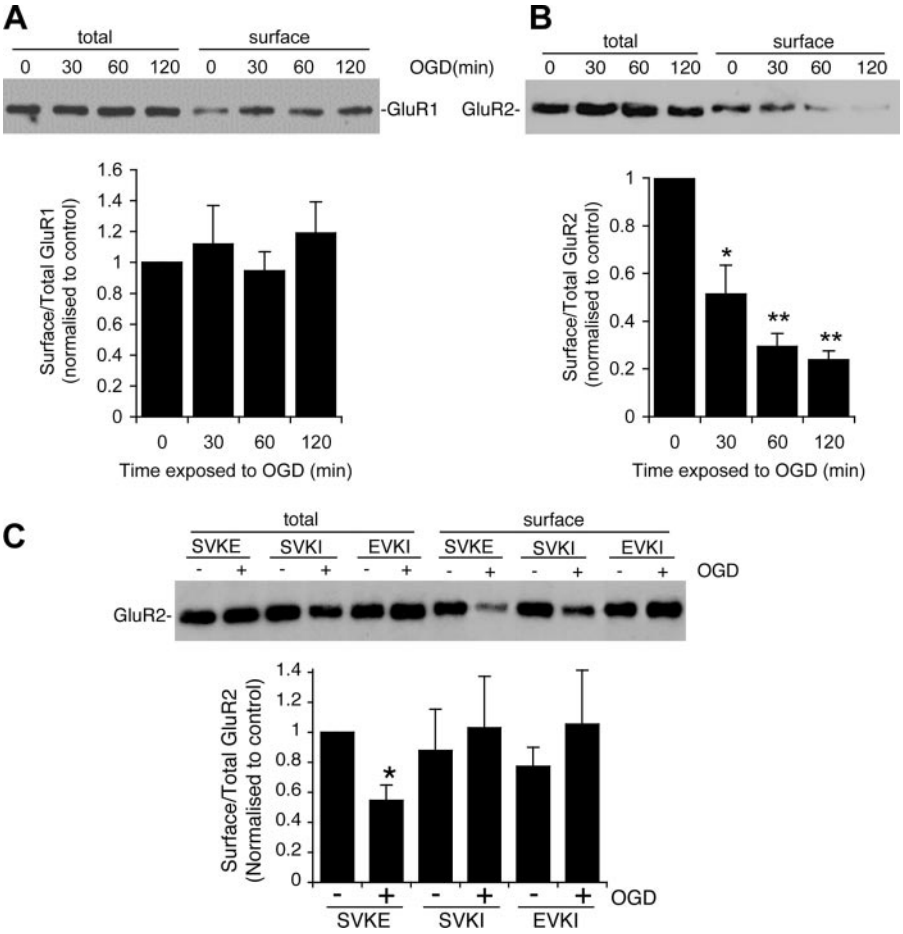

FIGURE 3. **OGD induces a rapid, PICK1-dependent removal of GluR2 from the surface of hippocampal neurons.** *A*, GluR1 surface levels are unaffected by OGD. Dissociated hippocampal neurons were exposed to OGD for the times indicated followed by biotinylation of surface proteins. The *top panel* shows a representative Western blot of total GluR1 present in lysates and surface (biotinylated) GluR1 after OGD. The graph shows pooled data presented as ratios of surface over total GluR1. $n = 5$. *B*, GluR2 is rapidly removed from the surface in response to OGD. Methods are same as for *A*, above, except that the Western blot was probed for GluR2. $n = 5$, *, $p < 0.01$, **, $p < 0.001$. *C*, OGD-induced internalization of GluR2 is blocked by peptides that disrupt PICK1 PDZ domain interactions. Dissociated hippocampal neurons were transduced with Sindbis virus to express pep2-SVKE, pep2-SVKI, or pep2-EVKI. Cultures were exposed to OGD and analyzed as in *B*, above. $n = 5$, *, $p < 0.005$.

domains of PICK1 or of ABP and GRIP1 (hereafter referred to as ABP/GRIP). The wild-type sequence pep2-SVKI blocks PICK1 and ABP/GRIP PDZ domains, pep2-EVKI specifically blocks PICK1, and pep2-SVKE is an inactive control peptide (28). These peptides were included separately in our recording solution and diffused into the cell during the experiment. As expected, inclusion of the control peptide pep2-SVKE had no effect on the change in the rectification index induced by OGD (Fig. 2*A*, pre-OGD = 0.4 ± 0.04, post-OGD = 0.3 ± 0.03, $n = 7$, $p < 0.005$). However, the PICK1-specific pep2-EVKI completely blocked the decrease in the rectification index (Fig. 2*B*, pre-OGD = 0.33 ± 0.02, post-OGD = 0.36 ± 0.02, $n = 6$, $p = 0.46$). Surprisingly, pep2-SVKI, which blocks both PICK1 and ABP/GRIP PDZ domains, did not completely block the decrease in the rectification index (Fig. 2*C*, pre-OGD = 0.42 ± 0.04, post-OGD = 0.37 ± 0.03, $n = 6$, $p < 0.05$).

The inclusion of pep2-EVKI or pep2-SVKI in the intracellular solution has previously been shown to produce changes in the EPSC amplitude in the absence of OGD, although rectification changes were not tested (21, 28). We performed time-matched control experiments for each of the peptides used that were identical to the experiments described above, except for the absence of OGD. None of these controls produced changes in the rectification index (pep2-SVKE, pre-OGD = 0.34 ± 0.01, post-OGD = 0.31 ± 0.03, $n = 6$, $p = 0.21$; pep2-EVKI, pre-OGD = 0.39 ± 0.03, post-OGD = 0.38 ± 0.02, $n = 6$, $p = 0.57$; pep2-SVKI, pre-OGD = 0.44 ± 0.04, post-OGD = 0.42 ± 0.04, $n = 6$, $p = 0.48$), indicating that the inclusion of the peptides on their own did not alter AMPAR-EPSC rectification.

The OGD-induced Reduction in Surface-expressed GluR2 Is PICK1-dependent—To directly analyze the surface AMPAR subunit switching induced by OGD, we carried out surface biotinylations in dissociated hippocampal cultures. Neurons were subjected to OGD followed immediately by biotinylation of surface proteins. We observed a time-dependent reduction in GluR2 surface expression by OGD (Fig. 3*B*), which is consistent with a previous study (17). 30 min of OGD was sufficient to induce this trafficking event, with the maximal effect seen at 120 min. GluR1 surface expression was unaffected by OGD at all time points tested (Fig. 3*A*), as demonstrated previously (17). To investigate the role of PICK1 PDZ interactions, we used the Sindbis virus to express GluR2 C-terminal peptides in cultured neurons. The viruses are bicistronic to express enhanced green fluorescent protein in addition to the peptides. By visualizing green fluorescent protein fluorescence, we consistently observed that 80–90% neurons were infected. We then assayed surface GluR2 following a 30-min period of OGD. The control peptide pep2-SVKE had no effect on OGD-induced GluR2 trafficking. However, pep2-SVKI and pep2-EVKI, both of which disrupt PICK1 PDZ interactions, completely blocked the reduction in surface-expressed GluR2 in response to OGD (Fig. 3*C*). These data strongly suggest that PICK1 plays a crucial role in the OGD-induced switch in surface AMPAR subunit composition.

Activity of GluR2-lacking AMPARs during OGD Insult Contribute to Delayed Neuronal Death—To investigate the functional relevance of PICK1-mediated subunit switching during the OGD insult, we used NASPM to specifically block GluR2-lacking AMPARs. We applied the drug before OGD and washed it out immediately following the insult so that GluR2-

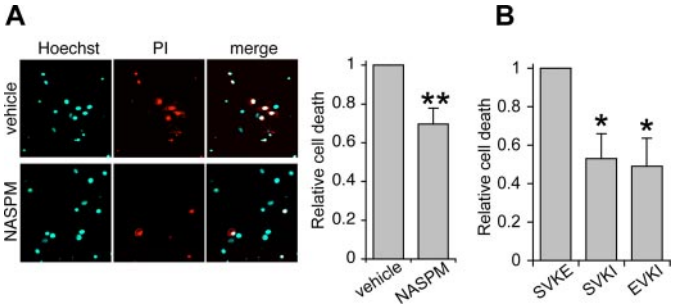

FIGURE 4. **PICK1-mediated switching of AMPAR subunit composition during OGD contributes to OGD-induced neuronal death.** *A*, blockade of GluR2-lacking receptors during OGD significantly reduces neuronal death. Dissociated hippocampal neurons were exposed to 45 min of OGD in the absence or presence of the GluR2-lacking AMPAR blocker NASPM (30 μM). Coverslips were washed extensively before returning them to normal conditioned growth medium. 30 h later, neurons were stained with Hoechst and PI, and the proportion of nuclei stained with PI was counted. The *left panels* show representative images for vehicle and NASPM-treated cultures. The graph shows pooled data, normalized to the vehicle-treated control. $n = 7$, **, $p < 0.01$. *B*, blockade of PICK1 PDZ domain interactions significantly reduces neuronal death. Dissociated hippocampal neurons were transfected with plasmids encoding the peptides pep2-SVKE (control), pep2-SVKI (wild type), and pep2-EVKI (PICK1-specific). Cultures were exposed to 45 min of OGD and stained with Hoechst and propidium iodide 30 h later, and the proportion of nuclei stained with PI was counted. Data are normalized to the pep2-SVKE condition. $n = 4$, *, $p < 0.05$.

lacking AMPARs would be blocked specifically during OGD. Cell death was then analyzed by PI staining 30 h later. NASPM application during OGD resulted in a significant reduction in delayed cell death (Fig. 4*A*), indicating that channel activity of GluR2-lacking AMPARs during OGD contributes to OGD-induced cell death.

PICK1-dependent Subunit Switching during OGD Insult Contributes to Delayed Neuronal Death—To specifically study the role of PICK1-mediated trafficking, we transfected hippocampal neurons with plasmids encoding GluR2 C-terminal peptides to block PICK1 PDZ domain interactions, exposed the cultures to OGD, and quantified delayed cell death by PI staining. Both the wild-type pep2-SVKI and the PICK1-specific pep2-EVKI significantly rescued neurons from OGD-induced cell death when compared with the inactive control peptide, pep2-SVKE (Fig. 4*B*). This strongly suggests that PICK1 PDZ domain interactions, and therefore PICK1-mediated AMPAR trafficking, are required for delayed OGD-induced cell death.

DISCUSSION

In this study, we have identified the GluR2-PICK1 interaction as a crucial mediator of OGD-induced AMPAR trafficking that results in an increased proportion of synaptic GluR2-lacking receptors. Our work also defines this trafficking event during OGD as a critical step leading to delayed neuronal death following the insult. In a previous study, it was shown that GluR2-PICK1 binding increases in hippocampal neurons following OGD (17); however, a causal link between this interaction and the switch in AMPAR subunit composition was not demonstrated. We have taken this further by providing direct evidence that disrupting PICK1-GluR2 interactions blocks the internalization of GluR2 from the cell surface and the resulting rectification change of synaptic AMPAR-EPSCs. Furthermore, we have demonstrated that PICK1-mediated regulation of GluR2 occurring during OGD is required for delayed neuronal death.

In addition to PICK1, the GluR2 PDZ ligand binds GRIP1 and ABP/GRIP. Our biotinylation data and cell death assays indicate that interfering with both PICK1 and ABP/GRIP PDZ domains (using pep2-SVKI) has the same effect as blocking just PICK1 (pep2-EVKI), suggesting that PICK1 is the more important protein. However, in our electrophysiology experiments, pep2-SVKI is less potent at blocking the OGD-induced rectification change when compared with pep2-EVKI. A possible explanation for this result may be that ABP/GRIP has an influence on GluR2-containing AMPARs that opposes that of PICK1. Consistent with this idea, it has been demonstrated that during OGD, the GluR2-PICK1 interaction is enhanced, whereas GluR2-ABP is reduced (17). The difference between the electrophysiology and cell biology results is likely to reflect the difference in mode of presentation of the peptide: acute infusion when compared with long term expression. The lack of an interfering peptide that is specific to ABP/GRIP PDZ domains makes it difficult to assess the role of ABP/GRIP using this approach.

It is well established that GluR2 mRNA levels and protein expression are reduced in hippocampal CA1 neurons 24–48 h after ischemia (29). The specific role of GluR2-lacking AMPARs brought about by PICK1-mediated trafficking at an earlier stage during OGD when compared with those expressed at later time points via altered gene expression is unclear. It is possible that the role of the early AMPAR-mediated calcium signal during OGD is to provide a trigger for signaling pathways that result in additional synaptic changes at a later time point, which may include altered *GluR2* gene expression.

A change in AMPAR-EPSC rectification has recently been described following LTP induction in CA1 hippocampal neurons (30) (but see Ref. 31), which may be PICK1-dependent (32). It has been proposed that the calcium influx through these GluR2-lacking AMPARs is required for stabilization of LTP, and therefore, for longer term changes in synaptic transmission. In the case of LTP, the rectification change is transient, lasting only for around 20 min following stimulus (30). However, the OGD-induced change in AMPAR subunit composition persists for at least 24 h after insult (17), suggesting that important functional as well as mechanistic differences exist between OGD and LTP. In our experiments, the increased AMPAR-EPSC rectification seen immediately after OGD is relatively small (~20% change) when compared with that seen during LTP expression (~80% change). During OGD, therefore, the resulting calcium influx via these early GluR2-lacking AMPARs is likely to be small yet sustained, whereas during LTP, the AMPAR-mediated calcium signal may be large and more transient (30). These precise temporal characteristics may be crucial in determining the downstream effects of AMPAR subunit rearrangements.

PICK1 has now been implicated in a number of physiological and pathological situations that involve regulating the GluR2 content of synaptic AMPARs. Interestingly, in most of these events, including during OGD (this study), PICK1 plays a role in restricting GluR2 from the synapse, rendering a proportion of AMPARs calcium-permeable (24, 33, 34). This is likely to

reflect a function for PICK1 in promoting GluR2 endocytosis or in restricting its recycling (35). However, in cerebellar stellate cells, PICK1 plays a role in mediating a switch from GluR2-lacking to GluR2-containing synaptic AMPARs (36, 37), suggesting that PICK1 can also promote forward traffic (or restrict endocytosis) of GluR2-containing AMPARs in certain cell types. Further work is needed to unravel the precise mechanisms that underlie how PICK1 specifically sorts and targets GluR2-containing AMPARs to appropriate subcellular locations.

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