Brief Communications

# The Transmembrane AMPA Receptor Regulatory Protein γ4 Is a More Effective Modulator of AMPA Receptor Function than Stargazin (γ2)

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**AMPA receptors mediate the majority of the fast excitatory synaptic transmission in the brain. A family of recently described auxiliary proteins, the transmembrane AMPA receptor regulatory proteins (TARPs) γ2, γ3, γ4, and γ8, have been shown to modulate the trafficking of receptors to the plasma membrane as well as electrophysiological key properties. Most studies published to date focus exclusively on γ2 (stargazin), neglecting the other three members of the TARP family. Here, we analyzed the modulation of electrophysiological properties of AMPA receptors by γ4 and compare it with γ2, using heterologous coexpression in human embryonic kidney 293 cells. We show for the first time that γ4, a previously poorly examined TARP, modulates the desensitization properties of AMPA receptors significantly stronger than γ2 does. In contrast, other properties such as kainate efficacy and current–voltage relationships are modulated in a similar way by both of these TARPs. From these TARP-specific effects, we propose an interaction mechanism between AMPA receptors and TARPs and address the physiological relevance of γ4 and its regulatory effects, particularly on AMPA receptor desensitization properties, to developmental and regulatory processes in the brain.**

*Key words:* **glutamate receptor; AMPA receptor; TARP; *stargazin*; γ4; desensitization**

## Introduction

Homomeric and heteromeric AMPA receptor channels are tetramers of the subunits GluR1–4 (Dingledine et al., 1999) that mediate the majority of fast excitatory synaptic transmission in the mammalian CNS. Interaction of AMPA receptors with proteins of the postsynaptic density (PSD) leads to insertion into and stabilization within the PSD as well as to dynamic regulation of synaptic AMPA receptors, which are involved in synaptic plasticity (Bredt and Nicoll, 2003).

A family of four such proteins interacting with AMPA receptors is defined by the transmembrane AMPA receptor regulatory proteins (TARPs). The first TARP discovered, γ2, is nonfunctional in the stargazer mouse (Letts et al., 1998). This lack of function of γ2 leads to an almost complete loss of AMPA receptor-mediated currents in cerebellar granule cells that can be rescued by exogenous γ2 (Hashimoto et al., 1999; Chen et al., 2000). The homologs of γ2, the proteins γ3, γ4, and γ8, were also able to rescue AMPA receptor-mediated currents, thereby defining the TARP family. Whereas all four TARPs show a distinct expression pattern in the adult CNS, γ4 is the only TARP expressed during early developmental stages (Tomita et al., 2003).

The interaction of γ2 with AMPA receptors results in two fundamentally different effects: an increased transport of AMPA receptors toward the plasma membrane followed by their insertion into the PSD (Chen et al., 2000) and an alteration of electrophysiological properties (Priel et al., 2005; Tomita et al., 2005; Turetsky et al., 2005). AMPA receptors interacting with γ2 show slowed desensitization and deactivation kinetics, as well as enhanced recovery from the desensitized state. Furthermore, the extent of desensitization is reduced, channel opening is prolonged, and the probability to reach high conductance opening levels is increased, as is the efficacy of the partial agonist kainate, leading to increased current amplitudes (Priel et al., 2005; Tomita et al., 2005; Turetsky et al., 2005). All these effects have been reported for γ2 and to a lesser extent for γ3. However, little is known about the TARPs γ4 and γ8. γ8 is expressed almost exclusively in the hippocampus where it is involved in the induction of long-term potentiation, thus underlying synaptic plasticity (Rouach et al., 2005). γ4, which is expressed diffusely in the adult brain, in both neurons and glial cells (Tomita et al., 2003), remains almost completely uncharacterized. In this study, we provide the first detailed characterization of the modulatory effects of γ4 on the electrophysiological properties of various homomeric and heteromeric AMPA receptor complexes. We show that the modulatory effects of γ4 on the extent and kinetics of AMPA receptor desensitization are far more pronounced than the respective effects of the well characterized γ2, whereas the kainate efficacy is influenced by both proteins in a similar manner.

## Materials and Methods

*Generation of constructs.* For expression in human embryonic kidney 293 (HEK293) cells, flip isoforms of rat AMPA receptor subunits were subcloned into pcDNA3.

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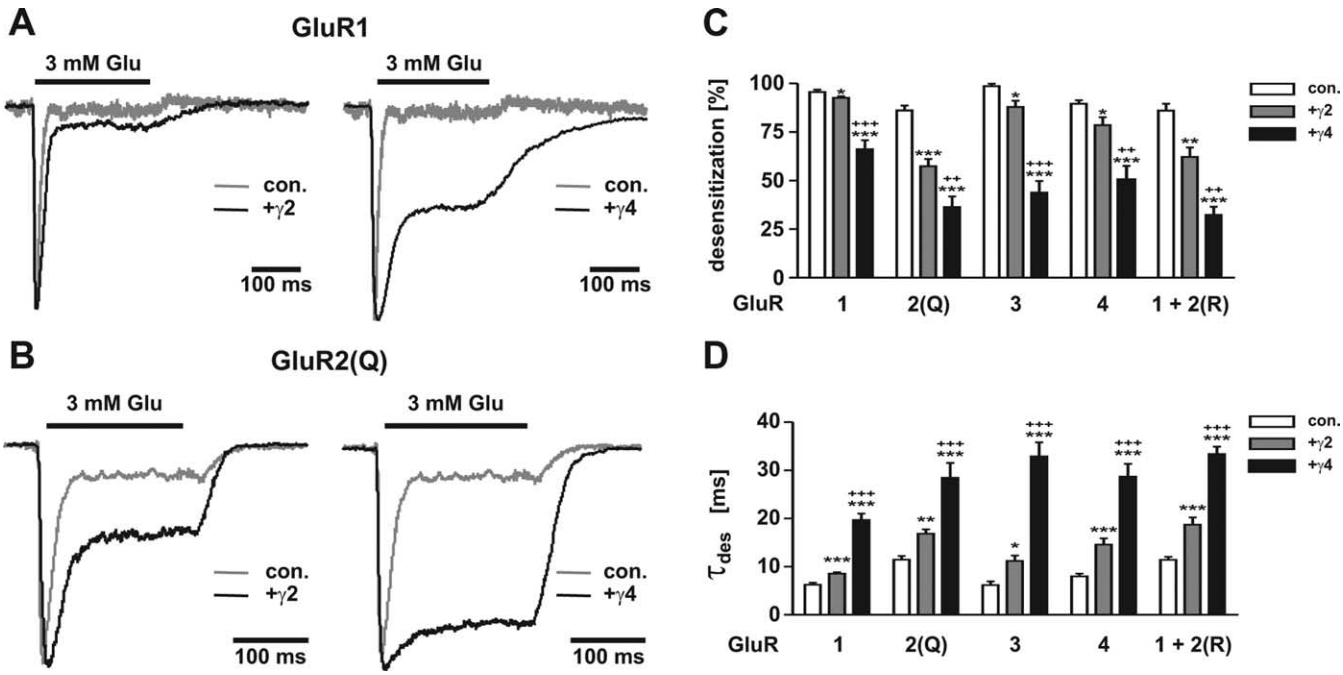


**Figure 1.** Modulation of the desensitization properties of AMPA receptors by γ4 is far more pronounced than by γ2. ***A***, ***B***, Representative scaled current responses of GluR1 (***A***) or GluR2(Q) (***B***) to the fast application of 3 mM glutamate, when coexpressed with either γ2 or γ4 (black traces) compared with current responses obtained in the absence of any TARP (gray traces). ***C***, Extent of desensitization of homomeric and heteromeric AMPA receptors in the absence of TARPs and during coexpression of either γ2 or γ4. ***D***, Desensitization time constants of homomeric and heteromeric AMPA receptors in the absence of TARPs or during coexpression of either γ2 or γ4. Bars represent means ± SEM ($n = 5$–30). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, significantly different from control (without TARP); $^{++}p < 0.01$, $^{+++}p < 0.001$, significantly different from coexpression of γ2. Con., Control.

Rat γ2 and γ4 were C-terminally tagged with enhanced cyan fluorescent protein (ECFP) by deletion of the stop codons by PCR and cloning of PCR amplimers of the complete coding regions of γ2 and γ4 into pECFP–N1 (Clontech, Palo Alto, CA) using unique *Sac*I and *Sal*I restriction sites. GluR1 was fused with enhanced yellow fluorescent protein (EYFP) by deletion of its stop codon and cloning into pEYFP–N1 (Clontech) using unique *Nhe*I and *Sal*I restriction sites.

*Heterologous expression.* HEK293 cells were cultured in Minimum Essential Medium Eagle, Joklik Modification (JMEM) (Sigma, Taufkirchen, Germany) containing 10% fetal bovine serum (FBS) (Invitrogen, Paisley, UK) at 37°C and 8% $CO_2$. For transfection, medium was changed to DMEM (Invitrogen) containing 10% FBS. Cells were cotransfected with cDNAs coding for AMPA receptor subunits and either an ECFP-tagged TARP or ECFP (at a 3:2 ratio) using the calcium phosphate method or Metafectene (Biontex, Martinsried, Germany). Transfection was performed for 8 h at 37°C, 3% $CO_2$. After transfection, the medium was changed back to JMEM.

*Electrophysiology.* Whole-cell patch-clamp recordings of HEK293 cells were performed 24–48 h after transfection at room temperature (21–24°C) using an EPC-9 amplifier (HEKA Elektronik, Lambrecht, Germany). Currents were digitized with a sampling rate of 10 kHz and filtered at 3 kHz. Pipettes were pulled from borosilicate glass and had resistances of 4–8 MΩ. The extracellular solution contained 140 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES adjusted to pH 7.3 with NaOH. The pipette solution contained 130 mM CsF, 33 mM KOH, 4 mM NaCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 11 mM EGTA, and 10 mM HEPES adjusted to pH 7.3 with KOH. Drugs were prepared in extracellular solution. Rapid (<1 ms) application of agonist was performed using a two-channel theta glass capillary (channel diameter, 0.23 mm) mounted on a piezoelectric translator. Time constants were calculated by single-exponential fits using Pulse Fit 8.7 (HEKA Elektronik). The extent of desensitization was calculated as the ratio between steady-state and peak glutamate-evoked currents, expressed as percentage of peak current $[(1 - I_{\text{steady state}}/I_{\text{peak}}) \times 100]$. Only cells with leak currents below 100 pA were analyzed. Cells with rise times indicating suboptimal perfusion were excluded. Significance was tested by two-tailed unpaired *t* tests.

## Results

### Desensitization properties of AMPA receptors are more strongly affected by γ4 than by γ2

We examined the modulatory influence of γ4, a previously little-characterized member of the TARP family, on the desensitization properties of AMPA receptors. The well characterized stargazin (γ2) was analyzed in parallel for comparison. We coexpressed either a TARP C-terminally tagged with ECFP or ECFP alone with the AMPA receptor subunit GluR1 in HEK293 cells and performed whole-cell patch-clamp measurements using a fast perfusion system. Relative expression levels of γ2 and γ4 compared with GluR1 were determined by fluorescence intensity analyses to be nearly equal (supplemental Fig. S1, available at www.jneurosci.org as supplemental material). Consistent with literature data (Priel et al., 2005; Tomita et al., 2005; Turetsky et al., 2005), the coexpression of GluR1 and γ2 led to a decrease in the extent and a slowing of receptor desensitization (Fig. 1*A*,*C*,*D*). Surprisingly, the coexpression of γ4 with GluR1 affected both properties to a much larger extent (Fig. 1*A*,*C*,*D*). The extent of desensitization of the receptor was decreased from 95.6 ± 1.2% in the absence of TARPs to 66.1 ± 4.7% during coexpression with γ4. This is a 10-fold larger effect than the γ2-induced reduction to 92.6 ± 0.8% receptor desensitization. In parallel, the desensitization time constant of GluR1 increased from 6.24 ± 0.43 to 19.68 ± 1.33 ms when coexpressed with γ4 compared with only 8.55 ± 0.31 ms with γ2 (Table 1).

The part of the glutamate-evoked peak currents we missed because of temporal resolution limits of the whole-cell configu-

ration was estimated from coapplication of glutamate (3 mM) and the desensitization blocker cyclothiazide (CTZ) (300 μM). Using the corrected peak currents, we recalculated the extent of desensitization for GluR1 to 98.4 ± 0.4% compared with 95.6 ± 1.2% for the uncorrected peak currents.

To test whether these γ4 effects were AMPA receptor subunit-specific or general γ4 properties, we coexpressed γ2 and γ4 with GluR2(Q), the engineered unedited form of GluR2 (Fig. 1*B*). The desensitization properties of GluR2(Q) were affected even more strongly than those of GluR1 by interaction with the two TARPs (Fig. 1*C*). The extent of desensitization was reduced to 57.4 ± 3.7% when coexpressed with γ2, in line with previously reported results (Turetsky et al., 2005). Nevertheless, the impact of γ4 was significantly stronger, dropping the extent of desensitization to 36.4 ± 5.5% (Table 1). Thus, for GluR2(Q), as for GluR1, γ4 has a much stronger impact on the extent of desensitization than γ2, although the difference is smaller than seen for GluR1. We conclude that the impact of γ4 on the extent of desensitization depends on the interacting AMPA receptor subunit, as was reported for γ2 (Turetsky et al., 2005).

The kinetics of receptor desensitization of GluR2(Q) were also strongly affected by both γ2 and γ4. Again, γ4 had much more pronounced effects than γ2. Desensitization time constants of GluR2(Q) were determined as 28.44 ± 3.10 and 16.84 ± 0.92 ms in the presence of γ4 and γ2, respectively, compared with 11.44 ± 0.78 ms obtained for GluR2(Q) in the absence of TARPs (Table 1). Concerning desensitization kinetics, no AMPA receptor subunit-dependent effect was observed (Fig. 1*D*). The slowing of desensitization induced by γ4 was twofold larger than that caused by γ2 in both GluR2(Q) and GluR1.

Given these two partly AMPA receptor subunit-dependent effects of γ2 and γ4, we examined whether the AMPA receptor subunits GluR3 and GluR4 were modulated by γ2 and γ4 in a similar manner. Although the extent of desensitization as well as the desensitization kinetics of GluR3 and GluR4 were strongly affected by both γ2 and γ4, the impact of γ4 was always larger.

The reduction by γ2 and γ4 of the extent of desensitization depended on the coexpressed AMPA receptor subunit: for GluR3 and GluR4 coexpressed with γ4, it was threefold to fivefold larger than that obtained in the presence of γ2 (Table 1, Fig. 1*C*). The modulation of the extent of desensitization differed substantially between AMPA receptor subunits and, in addition, varied with the coexpressed TARP. The kinetics of desensitization also varied with the AMPA receptor subunits but were similar for the TARPs γ2 and γ4 (Table 1, Fig. 1*D*). The rise times of the different AMPA receptors in the absence of TARPs and in the presence of γ2 ranged between 4.85 ± 0.4 and 11.09 ± 0.69 ms. In contrast, γ4 induced a prominent slowing of receptor activation for each of the examined AMPA receptors, resulting in rise times between 9.9 ± 0.9 and 20.9 ± 5.1 ms (data not shown).

### Kainate efficacy at AMPA receptor subunits is modulated similarly by γ4 and γ2

Because it had been reported that the kainate efficacy is increased by coexpression of γ2 for all AMPA receptor subunits (Turetsky et al., 2005), we investigated apparent kainate efficacies in the presence of γ4 for comparison.

Apparent kainate efficacies were calculated as the ratio of the kainate-evoked (600 μM) and glutamate-evoked (3 mM) peak currents and were low (0.04 ± 0.01 to 0.15 ± 0.03) in the absence of TARPs. Coexpression of γ2 led to a dramatic increase to values of above one for all AMPA receptor subunits (data not shown). γ4, in contrast, led to kainate efficacies of only 30–80% of those

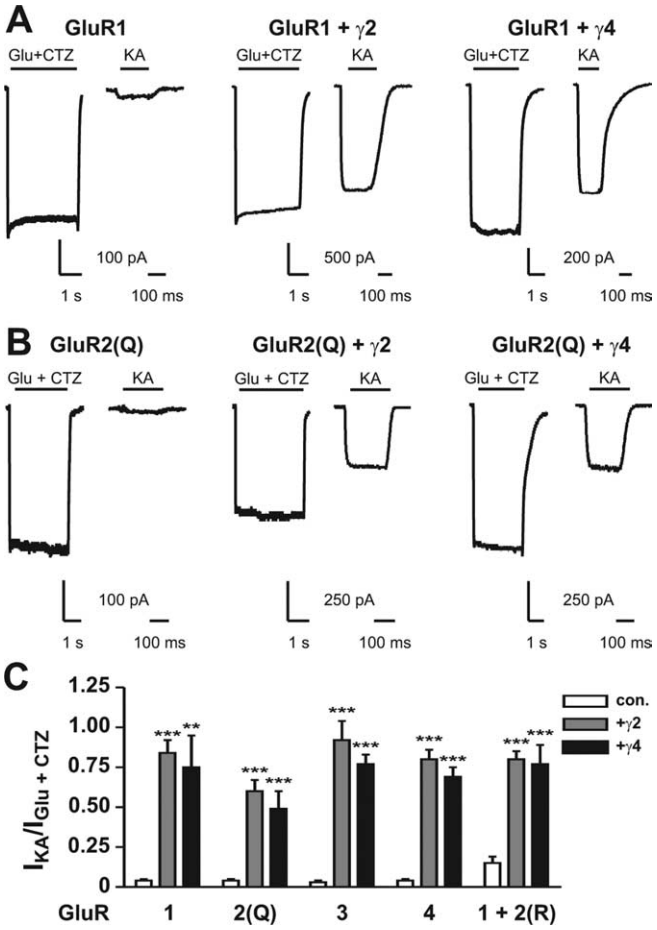


**Figure 2.** The kainate efficacy of AMPA receptors is modulated by γ2 and γ4 in the same manner. ***A***, ***B***, Representative current responses of GluR1 (***A***) or GluR2(Q) (***B***) during application of either glutamate (Glu; 3 mM) in the presence of CTZ (300 μM) (left traces) or kainate (KA; 600 μM) (right traces), in the absence of TARPs and in coexpression with either γ2 or γ4. ***C***, Apparent kainate efficacies of AMPA receptor complexes determined as ratios of $I_{KA}$ and $I_{Glu+CTZ}$. Bars represent means ± SEM ($n = 4$–10). $^{**}p < 0.01$, $^{***}p < 0.001$, significantly different from control (without TARP). Con., Control.

obtained in the presence of γ2. Remarkably, the differences between the kainate efficacies in the presence of γ2 and γ4 correlated with differences in the rise times of glutamate-evoked currents. This led us to suspect that we were missing a significantly larger part of the glutamate-evoked peak currents during coexpression of γ2 than of γ4, probably attributable to slower rise times in the presence of γ4. To test this hypothesis, we blocked receptor desensitization during glutamate application (3 mM) by coapplication of 300 μM CTZ and compared currents with those obtained during kainate application in the absence of CTZ, calculating the apparent kainate efficacy as $I_{KA}/I_{Glu+CTZ}$. AMPA receptor subunits in the absence of TARPs showed kainate efficacies only slightly lower than those obtained without CTZ. In contrast, kainate efficacies obtained in the presence of γ2 were reduced to ~0.8 for all AMPA receptor subunits. Only GluR2(Q) showed a slightly lower kainate efficacy of 0.6 ± 0.07 (Table 1, Fig. 2), consistent with results reported previously (Turetsky et al., 2005). The apparent kainate efficacies in the presence of γ4 of all AMPA receptors were similar to those in the presence of γ2 (Fig. 2*A*,*C*, Table 1). This finding suggests that the pronounced apparent differences in the kainate efficacies during coexpression of γ2 and γ4 in the absence of CTZ were attributable to technical limitations in the resolution of glutamate-induced peak currents

**Table 1. Modulatory effects of coexpression of either γ2 or γ4 on glutamate-evoked (3 mM) peak amplitudes, desensitization properties, and the kainate efficacy of AMPA receptors**

| | *n* | $I_{Glu}$ peak (pA) | $\tau_{des}$ (ms) | Extent of desensitization (%) | Apparent kainate efficacy |
|---|---|---|---|---|---|
| GluR1 | | | | | |
| Control | 9–20 | 210.1 ± 55.8 | 6.24 ± 0.43 | 95.6 ± 1.2 | 0.04 ± 0.01 |
| +γ2 | 9–30 | 745.5 ± 141.7 | 8.55 ± 0.31 | 92.6 ± 0.8 | 0.84 ± 0.08 |
| +γ4 | 7–22 | 925.8 ± 160.2 | 19.68 ± 1.33 | 66.1 ± 4.7 | 0.75 ± 0.20 |
| GluR2(Q) | | | | | |
| Control | 7–8 | 249.8 ± 34.9 | 11.44 ± 0.78 | 86.1 ± 2.5 | 0.04 ± 0.01 |
| +γ2 | 6–17 | 625.8 ± 90.8 | 16.84 ± 0.92 | 57.4 ± 3.7 | 0.60 ± 0.07 |
| +γ4 | 8–14 | 468.0 ± 90.5 | 28.44 ± 3.10 | 36.4 ± 5.5 | 0.49 ± 0.11 |
| GluR3 | | | | | |
| Control | 5–7 | 47.2 ± 6.6 | 6.20 ± 0.75 | 98.6 ± 1.3 | 0.03 ± 0.01 |
| +γ2 | 6–8 | 108.5 ± 24.6 | 11.20 ± 1.10 | 87.9 ± 3.2 | 0.92 ± 0.12 |
| +γ4 | 4–5 | 93.0 ± 28.9 | 32.88 ± 2.92 | 43.8 ± 6.0 | 0.77 ± 0.06 |
| GluR4 | | | | | |
| Control | 8–10 | 458.5 ± 188.0 | 7.98 ± 0.56 | 89.5 ± 1.9 | 0.04 ± 0.01 |
| +γ2 | 5–10 | 873.2 ± 213.7 | 14.58 ± 1.32 | 78.5 ± 4.1 | 0.80 ± 0.06 |
| +γ4 | 9–12 | 658.0 ± 149.0 | 28.67 ± 2.67 | 50.4 ± 7.2 | 0.69 ± 0.06 |
| GluR1/GluR2(R) | | | | | |
| Control | 8–10 | 250.0 ± 20.4 | 11.42 ± 0.63 | 86.0 ± 3.5 | 0.15 ± 0.04 |
| +γ2 | 6 | 276.5 ± 32.0 | 18.70 ± 1.53 | 62.2 ± 4.8 | 0.80 ± 0.05 |
| +γ4 | 5 | 390.5 ± 60.7 | 33.38 ± 1.53 | 32.3 ± 4.2 | 0.77 ± 0.12 |

Apparent kainate efficacy: ($I_{KA}/I_{Glu+CTZ}$) with KA at 600 μM, Glu at 3 mM, and CTZ at 300 μM. Extent of desensitization (%): $(1 - I_{Glu\ steady\text{-}state}/I_{Glu\ peak}) \times 100$, with Glu at 3 mM. Control, Without TARP.

especially in the presence of γ2. In summary, the interaction of AMPA receptor subunits with γ4 results in a dramatically increased kainate efficacy, similar to what has been reported for γ2 (Turetsky et al., 2005).

### Current–voltage relationships are slightly altered by coexpression of either γ2 or γ4

An important property of AMPA receptors is the shape of the current–voltage (*I–V*) relationship, which is inwardly rectifying for the Q variants and linear for edited R variants of AMPA receptors. *I–V* relationships were determined for all AMPA receptor complexes listed in Table 1 in the presence or absence of γ2 or γ4. *I–V* relationships were always inwardly rectifying and thus not changed by the coexpressed TARP. We determined the conductance ratios at +40 and −60 mV to vary between 0.01 ± 0.01 (GluR1; $n = 12$) and 0.13 ± 0.03 (GluR1 plus γ4; $n = 9$), clearly indicating inward rectification when compared with 0.68 ± 0.11 ($n = 6$) during coexpression of GluR1(Q) and GluR2(R).

At holding potentials above +30 mV, small outward currents were detected in coexpression experiments of all AMPA receptor subunits with γ2 or γ4. Additionally, the slope of the *I–V* relationships during coexpression of AMPA receptors with γ2 or γ4 flattened at negative holding potentials (data not shown).

### The effects of γ2 and γ4 on heteromeric and homomeric receptors are similar

Although homomeric AMPA receptors may occur in the brain (Wenthold et al., 1996), they are probably not the most common form of AMPA-type glutamate receptors. Therefore, we next examined heteromeric channels and coexpressed GluR1(Q) and GluR2(R) either alone or together with γ2 or γ4. The presence of both receptor subunits was confirmed by the linear *I–V* relationship caused by the coexpressed R variant subunit.

The extent and kinetics of desensitization of GluR1/GluR2(R) heteromeric receptor complexes were modulated similarly to homomeric receptors. Desensitization kinetics were slowed by γ2 and γ4, resulting in desensitization time constants of 33.38 ± 1.53 ms in coexpression with γ4 compared with 18.7 ± 1.53 ms with γ2 and 11.42 ± 0.63 ms in the absence of TARPs (Table 1, Fig. 1*D*). As reported for homomers above, receptor activation was slowed by coexpression of γ4.

The extent of desensitization of the receptor complex was reduced remarkably by coexpression of either γ2 or γ4; however, the effect of γ4 was twice as large as that of γ2, reducing receptor desensitization to only 32.3 ± 4.2% (Table 1, Fig. 1*C*) and extending our findings of differential TARP effects to heteromeric receptors.

Apparent kainate efficacies were determined for the GluR1/GluR2(R) receptor complex alone and in coexpression with γ2 or γ4. In the absence of CTZ, apparent kainate efficacies in the presence of γ4 were only half of those found during coexpression of γ2. When repeated in the presence of CTZ, coexpression of γ2 or γ4 resulted in similar increases in kainate efficacies, to 0.8 ± 0.05 and 0.77 ± 0.12, respectively, which is almost identical to the efficacies obtained for homomeric channels in the presence of TARPs (Table 1, Fig. 2*C*). However, the apparent kainate efficacies without TARPs were remarkably higher for heteromeric channels compared with homomeric channels, as has been reported previously (Turetsky et al., 2005).

Comparison of the *I–V* relationships of GluR1/GluR2(R) receptor complexes with and without coexpression of γ2 and γ4 did not reveal any differences in shape (data not shown); all *I–V* relationships were linear, with conductance ratios between 0.68 ± 0.11 [GluR1/GluR2(R); $n = 6$] and 0.73 ± 0.08 [GluR1/GluR2(R) + γ4; $n = 7$].

## Discussion

In the present study, we compared the modulatory effects of the TARPs γ4 and γ2 on homomeric and heteromeric AMPA receptor complexes. Both TARPs caused a reduction in the extent of desensitization and a slowing in desensitization kinetics. Whereas the impact on the desensitization kinetics of the receptor complexes only depended on the coexpressed TARP, the impact on the extent of desensitization additionally depended on the AMPA receptor. γ2 caused only a moderate decrease in the extent of desensitization and a relatively small increase in desensitization time constants for all AMPA receptors except GluR2(Q). Coexpression of γ4, in contrast, induced an up to 10-fold stronger

decrease in the extent of desensitization and a prominent slowing of desensitization kinetics. We also examined the expression levels of γ2 and γ4 relative to GluR1 and observed only minor differences, with γ4 having a slightly, but not significantly, higher relative expression. Nevertheless, these differences cannot explain the huge differences in effectivity.

γ2 had a strong impact on the extent of desensitization of all GluR2-containing receptor complexes, indicating a subunit-dependent effect and confirming previous studies (Yamazaki et al., 2004; Turetsky et al., 2005). However, the desensitization kinetics of GluR2 complexes were altered in the same way as the kinetics of other homomeric receptor complexes.

These findings can be correlated with the physiological occurrence of TARPs. γ4 is highly expressed during early development of the CNS (Tomita et al., 2003), a period critical for the formation of synapses and therefore synaptic plasticity. Mechanisms altering synaptic strength often rely on synaptic activity and calcium influx. Therefore, interaction of TARPs, particularly of γ4, with AMPA receptor complexes may lead to increased postsynaptic currents and thereby favor upregulation of synaptic strength.

In the adult brain, γ4 is diffusely expressed not only in neurons but also in glial cells (Tomita et al., 2003); in the latter, γ4 may induce regulatory processes that enhance synaptic activity. Such processes may include facilitation of glutamate uptake from the synaptic cleft in a calcium-dependent manner or signaling between neurons and glial cells as well as activity-induced gene transcription in glial cells (Gallo and Ghiani, 2000).

The apparent kainate efficacy was strongly increased in nearly all examined receptor/TARP combinations. GluR2(Q), which showed a strongly reduced and slowed desensitization during coexpression of either γ2 or γ4, showed the smallest increase in kainate efficacy of all tested combinations, suggesting that at least on the GluR2 subunit different interaction sites may be responsible for the modulation of kainate efficacy and desensitization properties.

The *I–V* relationships of AMPA receptor complexes were not altered noticeably by coexpression of γ4 or γ2. Only changes at extremely positive potentials could be detected, as has been reported previously for γ2 (Yamazaki et al., 2004; Turetsky et al., 2005).

**Possible role of AMPA receptor domains in TARP interaction**

Our data lead us to propose the following scheme of regulatory interaction between AMPA receptors and TARPs: until now, chiefly two regions in the AMPA receptor have been linked to the desensitization properties of the receptor, namely the ligand-binding domain (LBD) (Partin et al., 1995; Stern-Bach et al., 1998; Banke et al., 2001; Sun et al., 2002; Horning and Mayer, 2004) and the linker regions between the LBD and the pore region (Yelshansky et al., 2004). Hence, an interaction between the TARP, probably at the first extracellular loop (Tomita et al., 2005), and one of these two regions of the AMPA receptor seems likely.

An interaction of the TARP with the LBD may stabilize the dimer interface of the LBD, thereby reducing the extent of desensitization and slowing its kinetics. In this case, the same or a second TARP/AMPA receptor interaction would produce a more efficient closure of the binding cleft during kainate binding. Such an interaction between TARP and binding cleft could also be allosteric and does not have to be prominent, because even a point mutation in the cleft is sufficient to increase kainate efficacy strongly (Armstrong et al., 2003).

Conversely, an interaction between the TARP and the linker regions connecting the LBD and the pore region cannot be ruled out, especially because it has been shown that point mutations within a linker region influence the desensitization properties of the receptor complexes (Yelshansky et al., 2004). In this case, not only desensitization properties of the receptor complex could be altered but also the gating. This may explain the prolonged channel opening and increased probability to reach high conductance levels in the presence of γ2 as reported by Tomita et al. (2005). Additionally, despite the smaller amount of cleft closure during kainate binding compared with glutamate binding, kainate binding might be able to induce high conductance channel openings in the presence of interactions between TARPs and the linker regions. This could explain the increased kainate efficacy in the presence of TARPs. Such a model predicts at least two interaction sites, because otherwise it would be difficult to explain the differential effects of γ2 and γ4 on the desensitization properties and kainate efficacy of GluR2(Q). Because all three linkers are perfectly conserved among the AMPA receptor subunits, it seems more likely that the interaction between AMPA receptors and TARPs takes place at the LBD. However, an interaction via the linker region cannot be ruled out entirely because the observed subunit-dependent effects could potentially be induced allosterically.

In summary, we showed that a previously little-examined member of the TARP family, γ4, has a far greater impact on the desensitization properties of either homomeric or heteromeric AMPA receptor complexes than the well characterized γ2. These modulatory effects, in conjunction with the high expression levels of γ4 in early postnatal stages and its diffuse expression in adult brain, neurons, and glial cells, may underline the importance of γ4 for developmental processes in the CNS and for regulatory or communicational processes in the adult brain.

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