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Tarps differentially affect the pharmacology of ampakines

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ABSTRACT

Transmembrane AMPA receptor regulatory proteins (TARPs) govern AMPA receptor cell surface expression and distinct physiological properties including agonist affinity, desensitization and deactivation kinetics. The prototypical TARP, STG or γ2 and TARPs γ3, γ4, γ7 and γ8 are all expressed to varying degrees in the mammalian brain and differentially regulate AMPAR gating parameters. Positive allosteric AMPA receptor modulators or ampakines alter receptor rates of agonist binding/unbinding, channel opening and can offset receptor desensitization and deactivation. The effects of the two ampakines, CX614 and cyclothiazide (CTZ) were evaluated on homomeric GluR1-flip receptors and GluR2-flop receptors expressed on HEK293 cells by transient transfection with or without different TARPs γ2, γ3, γ4 or γ8 genes. γ4 was the most robust TARP in increasing the affinities of CX614 and CTZ on GluR1-flip receptors, but had no such effect on GluR2-flop receptors. However, γ8 gave the most significant increases in affinities of CX614 and CTZ on GluR2-flop. These data show that TARPs differentially affect the surface expression and kinetics of the AMPA receptor, as well as the pharmacology of ampakines for the AMPA receptor. The modulatory effects of TARPs on ampakine pharmacology are complex, being dependent on both the TARP subtype and the AMPA receptor subtypes/isoforms.

1. Introduction

The AMPA-glutamate receptor (AMPAR) is an ionotropic receptor that mediates the majority of fast excitatory synaptic transmission in the brain. AMPAR dysregulation has been implicated in multiple neurodegenerative and neuropsychiatric conditions, so understanding AMPAR physiology has become of paramount importance in translational neuroscience.

The differential expression of its subunits (GluR1-4), each with its own flip and flop variant, and assembly as a heterotetrameric complex in the synapse of different brain regions has been proposed as an explanation as to why different brain regions respond differently to quantal pulses of glutamate [1,2]. As such, there has also been considerable interest in proteins that either assist in the forward cycling of AMPAR subunits to the neuronal synapse [2,3], proteins that post-translationally modify AMPAR subunits to alter their physiological properties [4] and proteins that complex with the AMPAR at synapses and alter its structural properties and subsequent physiological characteristics.

Of the multiple proteins that alter AMPAR trafficking and overall function, transmembrane AMPAR regulatory proteins (TARPs) have been extensively studied over the past fifteen years and recognized as notable governors of AMPAR cell surface expression, gating kinetics [5–7], and mediators of AMPAR post-translational modifications [8]. Their expression in rat brains was determined to be brain region-dependent and age-dependent, with developmental switches in TARP expression occurring shortly after birth, in many cases [8]. TARPs have been shown to control synaptic plasticity and their transcriptional dysregulation have even been implicated in the development of neuropsychiatric diseases such as bipolar disorder [9] and schizophrenia [9,10].

Ampakines are a family of small molecules that act as positive modulators of AMPARs and are sub-categorized into 2 classes. Class 1 or high impact ampakines bind to the CTZ binding site at the dimer interface of opposing subunits and stabilize the agonist-bound, open conformation state. They do not exhibit direct agonistic effects, but modulate agonist binding affinity, desensitization and deactivation parameters [11]. Class II or low impact ampakines produce subtle, if any, effects on receptor desensitization, but primarily accelerate channel opening. TARPs interact with the outer portion of the AMPA receptor ligand binding domain [12] which experiences a conformational change upon ampakine binding to the dimer interface in the inner portion of the tetramer complex [13]. Thus, we previously set out to determine whether Stargazin (TARP γ2 or STG) co-expression altered

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the pharmacology of 2 well-characterized high impact ampakines. Our studies demonstrated that STG had profound effects on the pharmacology of ampakines, primarily by influencing their ability to interfere with glutamate induced desensitization. These effects of CTZ and CX614 were due to increases in the affinity of these ampakines flop and flip receptors, respectively [7]. In the current work, we extend our studies by examining the effects that TARPs γ3, γ4 and γ8 have on the pharmacology of CX614 and CTZ and examine their differential effects on ampakine on and off rate to glutamate-bound AMPAR subunits.

2. Methods

2.1. Plasmids

Plasmids containing the rat GluR1-flip (GluR1i) and GluR2-flop (GluR2o) receptor cDNAs in the pRK5 expression vector under the cytomegalovirus (CMV) promoter were kind gifts from Dr. Kathryn M. Partin (Colorado State University, Fort Collins, CO). Enhanced green fluorescent protein (pEGFP-C3) plasmids was purchased from Clontech. (Palo Alto, CA). Rat Cacng2 (Stargazin) gene or γ3, γ4, γ8 were subcloned in pcDNA 3 vector by Kelen Biolab (San diego , CA).

2.2. Transfection

HEK293 cells (ATCC, Manassas, VA) were grown in DMEM (Gibco/Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (Gemini, West Sacramento, CA), 100 U/mL penicillin and 0.1 mg/mL streptomycin (Sigma, St. Louis, MO) at 37 °C in 6% CO_2 incubator. One or two days before transfection, HEK293 cells were trypsinized and re-plated into 35 mm culture dishes at a density of 10–14 × 10^4 cells per dish. Transfections were performed using SuperFect (Qiagen, Valencia, CA). For experiments with GluR1-flip receptor alone, the DNA ratio of Glu1-flip: pEGFP was 1.0 μg:1.0 μg. For experiments with GluR1-flip receptor with TARPs, the ratio of Glu1-flip:TARP:pEGFP was 0.3 μg:0.3 μg:1.4 μg. The transfected cells were identified under fluorescence microscope. Electrophysiology experiments were conducted ~24 h after transfection for both experiments involving GluR1-flip. For experiments with GluR2-flop receptor alone, the ratio of Glu2-flop: pEGFP was 1.5 μg:0.5 μg and electrophysiology experiments were conducted ~48 h after transfection. For experiments with GluR2-flop receptor with TARP, the ratio of Glu2-flop: TARP: pEGFP was 0.65 μg:0.65 μg:0.65 μg and electrophysiology experiments were conducted ~24 h after transfection.

2.3. Electrophysiological recording

Prior to recording, a dish was removed from the incubator and the culture medium was replaced with recording saline containing (in mM): NaCl, 145; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 0.8; Hepes, 10; D-glucose, 10; sucrose 30, and tetrodotoxin 20 nM, titrated to pH 7.4 with NaOH. Whole-cell recordings were made at room temperature with pipettes pulled from 1.5-mm o.d. glass capillary tubes (WPI, Sarasota, FL) with a P-97 micropipette puller (Sutter Instruments, Novato, CA). Patch pipettes were filled with a solution containing (in mM): potassium gluconate, 140; $CaCl_2$, 0.1; EGTA, 1.1; $MgCl_2$, 5; Hepes, 20; ATP, 3; phosphocreatine, 3.0; and GTP, 0.3; pH 7.4. The cells were voltage-clamped at −80 mV.

All compounds or saline were applied by DAD-12 or DAD-VC superfusion system (ALA Scientific Instruments Inc., New York). The 13 to 1 or 17 to 1 tip was placed approximately 100–150 μm from the cell. Cells were pre-treated for 20 s with modulators/saline before the application of glutamate/kainate for 2 s. The mean value of plateau current between 1650 ms and 1950 ms after application of agonist with and without modulators/saline was measured and used as the parameter to evaluate the compound's EC50 values. Values are mean ± SE. Concentration-response data from individual neurons were normalized (see figure legend), and the mean normalized results were fitted with Hill-type functions to obtain EC_{50} values. Results were compared using a type 2, 2-tailed students *t*-test compared to appropriate vehicle controls (usually when a TARP was not co-transfected). Alpha value was set at $p = 0.05$.

Table 1
EC_{50} values (μM) of ampakines in specific TARP/AMPAR subunit interactions. Data are Mean(SEM) of 5–13 cells. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, *t*-test comparing EC_{50} values of ampakines in the presence and absence of TARP co-expression.

	Control	STG	TARP3	TARP4	TARP8
CX614/1i	1500 (68.9)	281 (5)***	377 (8)***	102 (12)***	231.5 (79.1)***
CX614/2o	16.6 (2.0)	10.8 (2.3)	11.6 (1.1)*	15.7 (2.8)	10.2 (1.1)**
CTZ/1i	63 (8.1)	49 (6.4)	54 (2.2)	35 (3.1)	48 (3.1)
CTZ/20	1000 (34.2)	390 (7.7)***	530 (15)***	560 (12)***	312 (21.1)***

3. Results

We began our studies by examining the effects TARP co-expression elicited on glutamate and kainate-induced currents on homomeric GluR1i AMPARs. As determined previously [7], in the absence of a TARP, glutamate induces a more robust steady-state and peak current than does kainate even though glutamate-induced currents partially desensitize (Fig. 1a). However, when any of the 4 TARPs were co-expressed, kainate induces a much larger current than does glutamate. Similarly, the kainate-mediated currents do not desensitize whereas the glutamate-induced currents desensitize to a similar extent. STG and TARP γ3 increase the ratio of kainate/glutamate induced currents 100-fold while TARPs γ4 and γ8 similarly increase the ratio ~15-fold, suggesting potentially 2 distinct modes of action for these TARPs (Fig. 1b, $p < 0.01$, *t*-test). Furthermore, the efficacy of kainate as an agonist was differentially augmented by TARP co-expression. Specifically, with STG co-expression, kainate alone elicited 43% of the current elicited by 500 μM glutamate and CTZ, to which the currents were normalized (Fig. 1c). The other TARPs differentially enhanced kainate efficacy, though not to the extent seen with STG.

Next, we asked whether TARP co-expression could modulate the pharmacology of CX614, a flop-preferring ampakine, for homomeric GluR1i AMPARs. In the absence of TARPs, 1 mM CX614 produced 40% of the current elicited by 500 μM and 300 μM CTZ (Fig. 2a). However, TARP co-expression profoundly reduced the EC_{50} of CX614. We again show that STG co-expression reduced the EC_{50} of CX614 significantly, ~5.3-fold ($p < 0.001$, *t*-test). More striking is that co-expression of γ4 reduced the EC_{50} of CX614 from ~1500 μM to 108 μM, a 14-fold difference (Fig. 2b). TARP γ8 similarly lowered the EC_{50} of CX614, to a higher extent than STG did but not to the extent of TARP γ4.

It is well documented that CX614 has a higher affinity for flop-containing AMPAR subunits than those containing flip variants. Thus, we were not surprised that TARP co-expression did not profoundly alter the pharmacology of CX614 on GluR1o subunits. TARP γ8 did significantly lower the EC_{50} of CX614 from 16.6 μM to 10.2 μM ($p < 0.01$, *t*-test, Fig. 3b). TARP γ3 produced a similar significant reduction in EC_{50} ($p < 0.05$, *t*-test). We then went on to examine the effects TARP co-expression had on and off rates of CX614. This was done by using receptors in which glutamate was already in recording solution. This assured that any increase in current would be due to the addition of an ampakine (hence desensitized receptors). Fig. 3c shows data where STG strongly reduces the on rate of CX614 but does not significantly change modulator off rate. STG and TARP γ3 significantly reduced the onset of CX614 (Fig. 3d, $p < 0.05$, *t*-test) while TARPs γ4 and γ8 significantly increased the onset of CX614 binding (Fig. 3d). Furthermore, TARPs γ4 and γ8 significantly slowed dissociation of CX614 from desensitized