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Novel potent AMPA analogues differentially affect desensitisation of AMPA receptors in cultured hippocampal neurons

Tue G. Banke [1], John D.C. Lambert [*]

PharmaBiotec Research Center, Department of Physiology, University of Aarhus, Universitetsparken 160, DK-8000 Århus C, Denmark

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Abstract

The agonist actions of two AMPA receptor analogues, (*RS*)-2-amino-3-(3-carboxy-5-methyl-4-isoxazolyl) propionic acid (ACPA) and (*RS*)-2-amino-3-(3-hydroxy-5-trifluoromethyl-4-isoxazolyl)propionic acid (Tri-F-AMPA) have been studied on cultured rat hippocampal neurons. Whole-cell recordings with semi-rapid application of the agonists were used to study steady-state (plateau) responses. ACPA was the most potent agonist (EC_{50}, 1.2 μM), followed by AMPA (4.3 μM) and Tri-F-AMPA (4.6 μM), corresponding to a potency ratio of 4:1:1. Hill coefficients were close to 1 for AMPA and ACPA and close to 2 for Tri-F-AMPA, respectively. Plateau responses to maximal concentrations of the three agonists varied more than 2-fold. ACPA responses were 2.1 times greater and responses to Tri-F-AMPA were 1.6 times greater than responses to AMPA, respectively. Peak responses and desensitization were studied by using a fast piezoelectric device to apply agonists rapidly to outside-out patches. The time constants of desensitization were 8 ms for AMPA, 12 ms for Tri-F-AMPA and 17 ms for ACPA. There were no significant differences in the time-to-peak and 10–90% rise-time of the responses. The results indicate that of the three agonists tested, ACPA is the most potent at AMPA receptors expressed in cultured hippocampal neurons and that the maximum response to the agonists is inversely related to the rate of desensitization.

Keywords: AMPA receptor; AMPA receptor agonist; Desensitization; Hippocampal neuron

1. Introduction

Fast excitatory synaptic transmission in the central nervous system is predominantly mediated by glutamate receptors of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtype (Bettler and Mulle, 1995). Four subunits of the AMPA receptors have been cloned: GluR1-4 (Hollmann and Heinemann, 1994). Homomeric and heteromeric channels assembled from GluR1, GluR3, and/or GluR4 subunits show high permeability to Ca^{2+}, whereas the Ca^{2+} permeability of channels containing the GluR2 subunit is low (Hollmann et al., 1991; Verdoorn et al., 1991). AMPA receptor subunits co-assembled with the edited GluR2 (arginine) subunit exhibit a linear or outwardly rectifying current–voltage (I–V) relationship, while recombinant receptors lacking the GluR2 (arginine) subunit exhibit strong inward rectification (Boulter et al., 1990; Keinänen et al., 1990; Herb et al., 1992).

In hippocampal principal neurons, AMPA receptors desensitize relatively slowly ($\tau_{desens.}$ ~10–15 ms) and exhibit low Ca^{2+}-permeability (Mayer and Westbrook, 1987; Colquhoun et al., 1992; Livsey et al., 1993; Hestrin, 1993; Jonas et al., 1994). In hippocampal interneurons on the other hand, AMPA receptors show faster desensitization ($\tau_{desens.}$ ~5 ms) and are several times more permeable to Ca^{2+} (Hestrin, 1993; Jonas et al., 1994; Koh et al., 1995; Geiger et al., 1995; Isa et al., 1996). Using the reverse transcriptase-polymerase chain reaction (RT-PCR), Bochet et al. (1994) have demonstrated that GluR2 subunits are not present in a population of cultured hippocampal neurons for which the receptors show marked Ca^{2+} permeability and inward rectification.

A number of structural analogues of AMPA have recently been described by our colleagues (Hansen and Krogsgaard-Larsen, 1990; Krogsgaard-Larsen et al., 1992). These analogues include (*RS*)-2-amino-3-(3-carboxy-5-methyl-4-isoxazolyl)propionic acid (ACPA) and (*RS*)-2-amino-3-(3-hydroxy-5-trifluoromethyl-4-isoxazolyl) propi-

* Corresponding author. Tel.: +45-89-42-27-84; Fax: +45-86-12-90-65; E-mail: jl@fi.au.dk

[1] Present address: The Royal Danish School of Pharmacy, Department of Pharmacology, Universitetsparken 2, DK-2100 Copenhagen, Denmark.

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onic acid (Tri-F-AMPA). Electrophysiological and receptor-binding experiments have demonstrated that these analogues are potent and selective agonists at native AMPA receptors (Krogsgaard-Larsen et al., 1985; Lauridsen et al., 1985; Madsen et al., 1992; Madsen and Wong, 1992). We have recently investigated the effect of these analogues on recombinant AMPA receptors expressed in *Xenopus* oocytes (Wahl et al., 1996; Banke et al., 1997). The purpose of the present study was to examine the effect of these agonists on natively expressed AMPA receptors in cultured hippocampal neurons. Whole-cell recording and a semi-rapid application system were used to study steady-state responses while outside-out patches using a rapid ('concentration jump') application system was used to study the kinetics of peak responses and the ensuing desensitization.

2. Materials and methods

2.1. Materials

2.1.1. Compounds

Tri-F-AMPA, AMPA and ACPA were synthesized as previously described (Krogsgaard-Larsen et al., 1985; Madsen et al., 1992; Madsen and Wong, 1992) and provided by Povl Krogsgaard-Larsen and Ulf Madsen (Royal Danish School of Pharmacy). All other reagents and chemicals were purchased from Sigma (St. Louis, MO, USA).

All culturing media and chemicals were purchased from Gibco, with the exception of 5′-fluoro-2′-deoxyuridine (FuDR), uridine and poly-D-lysine, which were purchased from Sigma.

2.2. Neuron culture

Neurons were cultured from the hippocampi of 17–18-day old embryos as described previously (Kristiansen et al., 1991). Cells ($0.5–1 \times 10^5$) were plated into 35-mm Petri dishes containing coverslips which had been pretreated with poly-D-lysine. Plating medium was based on minimal essential medium (MEM) with Earle's salts and with L-analyl-L-glutamine (Glutamax-1) instead of glutamine, to which the following were added: 10% heat inactivated horse serum, 10% foetal calf serum, 50 IU/ml penicillin, 50 μg/ml streptomycin. One day after plating, the medium was completely exchanged with feeding medium (2 ml). This had the same composition as the plating medium, except that the foetal calf serum was removed and the horse serum was reduced to 5%. The medium was replenished twice a week by exchanging 1 ml with fresh medium. When visual inspection showed a confluent background layer of cells (usually after 3–4 days), mitosis was inhibited with FuDR (15 μg/ml) plus uridine (35 μg/ml).

2.3. Electrophysiology

Hippocampal cultures were used between 10–25 days in vitro. A glass coverslip containing the culture was transferred to the recording chamber (milled from stainless steel with a quartz glass base) mounted on the stage of an inverted microscope (Nikon Diaphot 200) equipped with Nomarski optics where the individual neurons were viewed at ×200. The recording chamber contained 2–3 ml artificial balanced salt solution (ABSS), which was renewed by constant perfusion at 1 ml min^{-1} with extracellular solution (see below). Recordings were made at room temperature (20–24°C). Standard patch-clamp techniques (Hamill et al., 1981) were used to record from neurons in the whole-cell configuration and from patches in the outside-out configuration using an Axopatch 200A patch-clamp amplifier (Axon Instruments). The patch electrodes were manufactured on the morning of the experiment from 1.2 mm o.d. thin-wall borosilicate glass tubing (Clark Electromedical) using a model P-87 electrode puller (Sutter Instruments). Electrode tips had internal diameters of around 1 μm and resistances of 2–4 MΩ. The patch electrodes were filled with solution just before use.

The agonists were applied using a semi-rapid 12 channel application system (DAD-12, Adams and List). The micro-manifold consisted of 12 teflon-coated quartz tubes of 100 μm i.d. fused together with a single 200 μm i.d. tube, which was used for flushing with extracellular medium. The end of this multi-channel assembly was connected by a short piece of narrow bore silicone tubing to a final common outlet, consisting of a short (approximately 5 mm) piece of 100 μm i.d. quartz glass tube. This outlet was positioned 100–200 μm from the soma of the recorded neuron. Each of the 12 tubes was connected to a reservoir (5-ml syringe) to which positive pressure could be applied. The outlet of each reservoir was led through a solenoid valve to the micro-manifold. Operation of the valves, which was controlled by a 386 computer using DAD-12 software (Adams and List), resulted in flow of the test solution out of the micro-manifold and onto the neuron. Between the applications of compounds, the neuron was continually superfused with normal ABSS by gravity feed from the single 200-μm barrel. This prevented hydrodynamic perfusion artifacts, and also hastened recovery following application of the compounds. This perfusion was also controlled by a solenoid valve, which was turned off and on concurrently with the application of test substances. Application of solutions of different ionic strength to the open tip of a patch electrode from which the junction potential was monitored showed that complete exchange of the solution was achieved within 50–100 ms, depending on the pressure applied to the reservoir.

The agonists were usually applied for 15 s every 55 s. Responses did not usually show appreciable 'run-down' with whole-cell recording and, after establishment of a constant response level to a single concentration (typically

100 μM) of AMPA, the concentration–response relationship for AMPA and the other agonists was investigated.

Rapid application of agonists ('concentration-jump') to outside-out membrane patches isolated from cell somata was made with double-barrelled theta-glass tube (o.d., 2.0 mm; wall thickness, 0.3 mm; septum thickness, 0.12 mm; Hilgenberg, Germany). Normal extracellular medium flowed continuously through one of the barrels. The other barrel was connected via a manually operated multi-way tap to one of five reservoirs (10 ml syringes) containing AMPA and its analogues. The test solution was selected by manual operation of the tap. Because of their limited availability, the maximum concentrations of ACPA and Tri-F-AMPA used were 0.5 mM and 1 mM, respectively. To serve as a reference, 1 mM AMPA was always present. The drug-containing channel was thoroughly flushed through with the next solution before it was applied. The theta-glass tube was stepped using a piezo-electric element (Burleigh Instruments). By measuring the change in junction potential between two different buffer solutions, the exchange time was determined to be less than 500 μs.

Unless otherwise stated, a holding potential (V_h) of −60 mV was used, from which the agonists evoked inward currents ($E_{AMPA} \approx 0$ mV). Currents were filtered at 10 kHz, digitized and stored on-line using a pentium computer. The signals were also recorded on video tape for off-line analysis using a modified digital-audio processor (Sony PCM-701es). The responses were also plotted on a low fidelity chart recorder (Servogor, model 124) during the experiment.

2.4. Solutions

Extracellular solution (ABSS) in mM: NaCl 140, KCl 3.5, Na_2HPO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, glucose 10 and HEPES 10 (310 mosM l^{-1}). pH was adjusted to 7.35 at 22°C using NaOH. Tetrodotoxin (0.2 μM) was added to block spontaneous regenerative and synaptic activity. Activation of NMDA-operated ionophores was prevented by the inclusion of Mg^{2+} and omission of glycine from the ABSS.

Patch electrode solution in mM: CsCl 130, $CaCl_2$ 1, tetraethylammonium chloride 10, $MgSO_4$ 1, EGTA 10, Leupeptin 0.1, MgATP 2 and HEPES 10 (290–295 mosM l^{-1}). The pH was adjusted to 7.35 at 22°C using CsOH. In some of the outside-out patch experiments, better sealing was achieved by replacing 10 mM CsCl with 10 mM CsF.

2.5. Data analysis

Concentration–response curves for the agonists were constructed by measuring the maximal peak and plateau responses induced by increasing concentrations of agonist. Data from individual cells were fitted to the Hill equation:

$$I = I_{max} / \left[1 + \left(EC_{50} / [\text{agonist}]\right)^n\right]$$

where I is the steady-state current produced by the agonist. The parameters I_{max} (maximal steady-state current at infinite agonist concentration), n (the Hill coefficient) and EC_{50} (concentration of agonist producing 50% of I_{max}) were determined by an iterative least squares fitting routine for each neuron and the mean ± S.E.M. is reported (SigmaPlot, Jandel, USA). The decaying phase of the current activated in outside-out patches by a 200-ms application of AMPA or its analogues was fitted with a single exponential function (Patch and Voltage Clamp software, Cambridge Electronic Design, England), yielding the time constant of desensitization (τ_{decay}). The curve was fitted from just after the peak and for the following 100 ms. The decay-fitting analysis was cross-checked using software (NPM 048X) designed by Dr. S. Traynelis, Department of Pharmacology, Emory University, GA, USA.

All values are means ± S.E.M.

3. Results

3.1. Agonist responses of cultured hippocampal neurons in the whole-cell configuration

With the semi-rapid application system, all the agonists evoked inward currents with a transient peak decaying back to a plateau level (Fig. 1). For plateau responses, ACPA was the most potent analogue tested. The EC_{50} value for ACPA (1.2 ± 0.1 μM ($n = 5$)) was approxi-

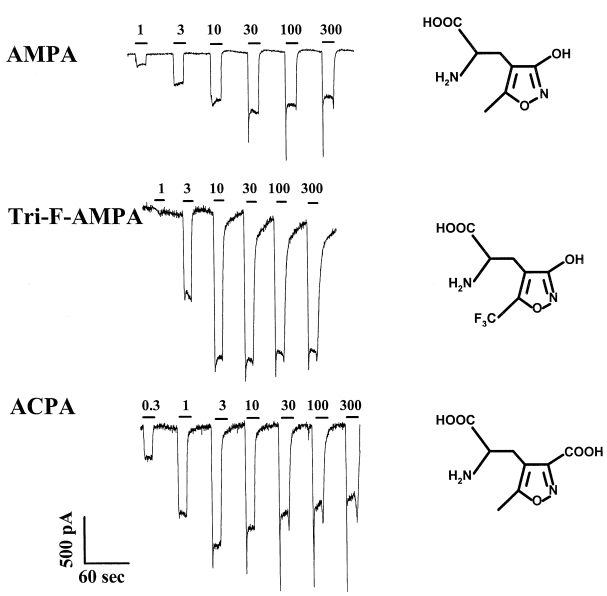

Fig. 1. Whole-cell recordings from three different cultured hippocampal neurons showing inward current responses evoked by AMPA (top trace), Tri-F-AMPA (middle trace) and ACPA (bottom trace) at a holding potential (V_h) of −60 mV. The agonists were applied by a semi-rapid application system at the concentrations (μM) given above with bars denoting the applications. The chemical structures of the respective analogues are shown on the right.

mately 4 times less than the EC_{50} values for AMPA (4.3 ± 0.4 μM ($n = 10$)) and Tri-F-AMPA (4.6 ± 0.1 μM ($n = 5$)) (Fig. 2A). The rank order of agonist potency was ACPA > AMPA ≥ Tri-F-AMPA. The values for the Hill coefficients were: for ACPA, 1.3 ± 0.1; for AMPA, 1.1 ± 0.1; and for Tri-F-AMPA, 2.0 ± 0.1.

3.1.1. Maximum responses to the agonists

To investigate the relative maximum response of the agonists, plateau responses evoked by 100 μM of each agonist were studied. This concentration evokes a maximal (or near-maximal) response for all three agonists (Fig. 2A). In order to control for differences in receptor expression between the neurons, responses were normalized to that evoked by a saturating concentration (100 μM) of AMPA. Marked differences in the amplitude of responses evoked by AMPA and its analogues were apparent and the maximum plateau responses varied more than 2-fold in amplitude (Fig. 2B). ACPA evoked the largest responses, which were $209 \pm 14\%$ ($n = 7$) of that to AMPA, while responses to Tri-F-AMPA were $161 \pm 15\%$ ($n = 7$) of that to AMPA. The sequence of maximum responses was thus: ACPA > Tri-F-AMPA > AMPA.

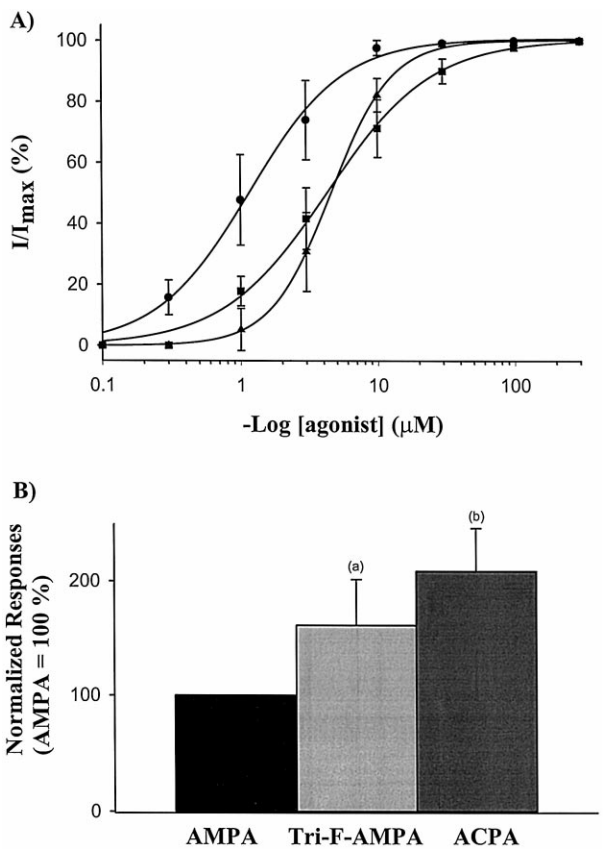

Fig. 2. A, Concentration–response curves for plateau responses evoked by ACPA (●), AMPA (■) and Tri-F-AMPA (▲) on cultured hippocampal neurons. Data points are expressed as a percentage (±S.E.M.) of the maximum plateau response for each agonist tested ($n = 6$–10 neurons). B, Comparison of the maximum responses to AMPA and its analogues. Whole-cell plateau responses were evoked by near-saturating concentrations (100 μM) of agonists and normalised with respect to the response evoked by 100 μM AMPA. Tri-F-AMPA and ACPA evoked significantly larger currents than AMPA ($n = 7$; a, $P < 0.01$; b, $P < 0.001$, paired *t*-test).

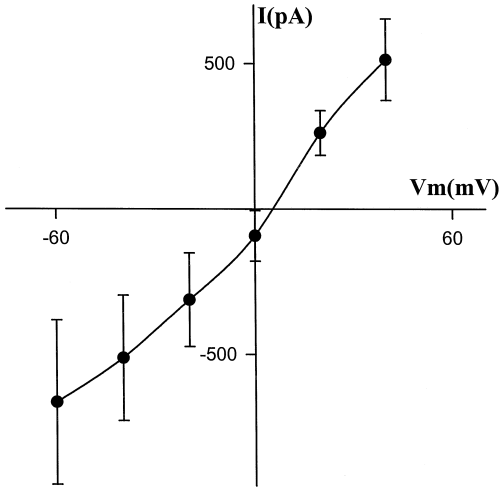

Fig. 3. Current–voltage (I–V) relationship for plateau response elicited by AMPA (30 μM) on hippocampal neurons ($n = 5$). I–V relationships were obtained by clamping the membrane at +20 mV increments from a holding potential of −60 mV and applying AMPA at each potential. The relationship is nearly linear with an interpolated reversal potential of around +5 mV.

3.1.2. I–V relationship

The I–V relationship of the plateau response to AMPA was linear in each of the five neurons in which it was investigated and the reversal potential was about +5 mV (Fig. 3). This would indicate that the AMPA receptors in this population of neurons contain GluR2 subunit(s) (Hume et al., 1991).

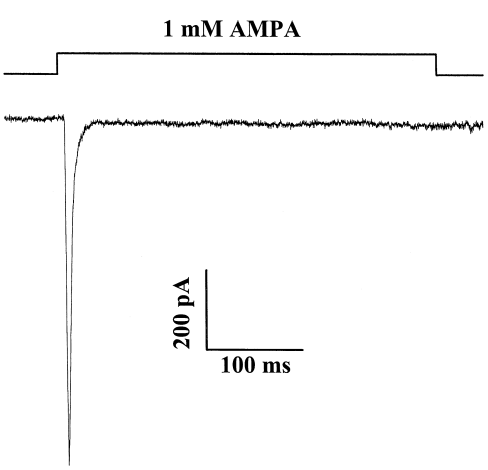

Fig. 4. Outside-out patch excised from a hippocampal neuron stimulated with 1 mM AMPA for 400 ms, as indicated by the command pulse to the piezoelectric unit in the upper trace. AMPA evoked a peak response of 840 pA which rapidly and completely desensitized within 25 ms. $V_h = -60$ mV.

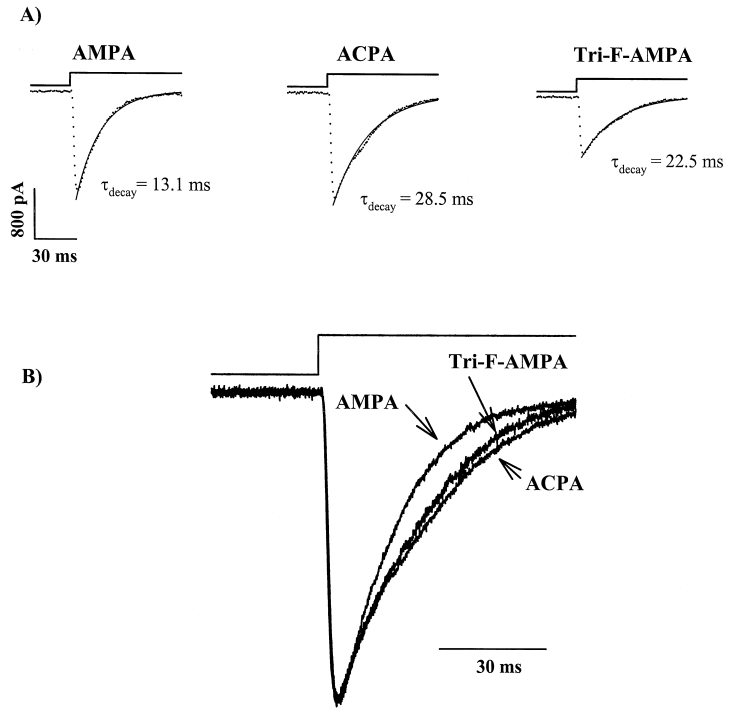

Fig. 5. Kinetics of the peak responses evoked by rapid applications of the AMPA analogues. (A) An outside-out patch excised from a hippocampal neuron and successively exposed to 1 mM AMPA, 0.5 mM ACPA and 1 mM Tri-F-AMPA. To control for run-down, the patch was exposed to 1 mM AMPA at the end of the experiment, where τ_{decay} was 14.2 ms (not shown). $V_h = -60$ mV. The command pulse to the piezoelectric system is indicated by the upper trace. The curves have been fitted by a mono-exponential function (full line) to give the τ_{decay} values indicated. (B) Traces from A normalised to the peak of the AMPA response and superimposed. The times-to-peak for the responses were: AMPA, 5.84 ms; ACPA, 3.65 ms; Tri-F-AMPA, 3.07 ms.

3.2. Peak responses in the outside-out configuration

Having established a whole-cell recording, the patch pipette was slowly withdrawn to form an outside-out patch. The response of patches to 1 mM AMPA (applied using the piezoelectric system) varied greatly and ranged from a few pA to approximately 1800 pA (Fig. 4). To ensure that the rate of desensitisation (τ_{decay}) was not affected by run-down, 1 mM AMPA was applied repetitively in several experiments. Although the peak amplitude of successive responses declined, normalisation to the same amplitude (cf. Fig. 5) showed that the decaying phases were identical (not shown) and were therefore not affected by run-down.

The amplitude and kinetics of the agonist-evoked responses were studied on the outside-out patches. As a reference, 1 mM AMPA was always applied in addition to Tri-F-AMPA (1 mM) and/or ACPA (0.5 mM) (Fig. 5). Three patches survived long enough to be stimulated with all three agonists, with responses to Tri-F-AMPA and ACPA being bracketed by AMPA applied at the beginning and the end of the recording to control for run-down. In most cases, the decaying phase could be fitted by a single exponential (Fig. 5). The values of τ_{decay} are given in Table 1, where it can be seen that the decaying phases of responses to Tri-F-AMPA and ACPA are significantly

Table 1

Agonist	τ_{decay} (ms)	*n*
AMPA	8±2	8
Tri-F-AMPA	12±3[b]	6
ACPA	17±5[a]	5

The rate of desensitization is agonist-specific.
τ_{decay} was determined by fitting the decaying phase of the agonist-evoked current recorded from outside-out patches with a single exponential function (cf. Fig. 5).
Significant differences from the AMPA response are indicated by letters ([a]$P < 0.05$; [b]$P < 0.01$, paired *t*-test).

Table 2

Agonist	Time-to-peak (ms)	10–90% Rise-time (ms)	*n*
AMPA	3.2±0.5	1.9±0.3	8
Tri-F-AMPA	2.1±0.3	1.7±0.1	6
ACPA	2.1±0.6	2.3±0.4	4

The kinetics of the initial phase of the peak response are independent of the agonist.
Each agonist-evoked response was fitted by linear regression to determine the time-to-peak.
The 10–90% rise-time of the response was measured over the linear portion of the rising phase.
There were no significant differences between the time-to-peak and 10–90% rise-time for the three agonists.

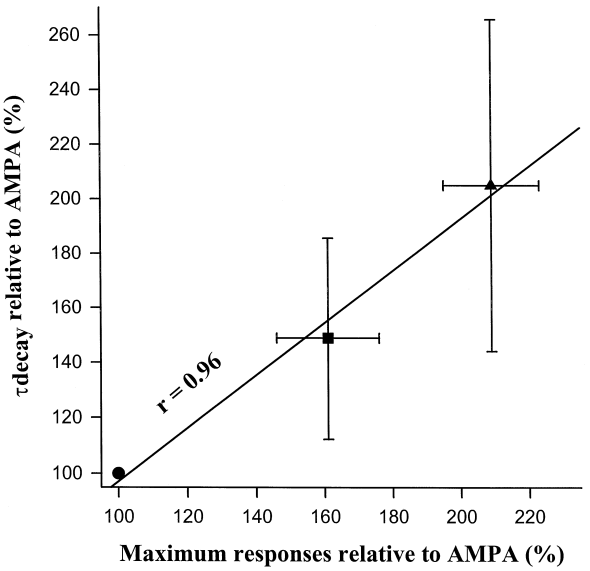

Fig. 6. Plot showing the relationship between maximum response and τ_{decay} for the three agonists (●, AMPA; ■, Tri-F-AMPA; ▲, ACPA). Values for maximum plateau responses and τ_{decay} are taken from Fig. 2B and Table 1, respectively, and are normalized to the response to AMPA. The line was drawn from a first order regression plot using SigmaPlot. Slope $(r) = 0.96$.

longer than those to AMPA. The time-to-peak and 10–90% rise time of responses to the three agonists were not significantly different (Table 2), although there was a tendency for the time-to-peak of responses to AMPA to be longer and for the rise time of responses to ACPA to be longer.

It was apparent that the ratios between the relative maximum response of the three agonists (Fig. 2B) and the τ_{decay} values (Table 1) were very similar. This strong correlation $(r = 0.96)$ is depicted in Fig. 6.

4. Discussion

4.1. Potency of the AMPA analogues

We have recently tested AMPA and its structural analogues on *Xenopus* oocytes injected with cDNA from either GluR1 or GluR3 and shown that the order of potencies was ACPA > Tri-F-AMPA > AMPA (Wahl et al., 1996; Banke et al., 1997). In the present investigation of native AMPA receptors expressed in cultured hippocampal neurons, the order of potencies was ACPA > AMPA ≥ Tri-F-AMPA. While ACPA was clearly the most potent analogue, AMPA and Tri-F-AMPA were equipotent for plateau responses.

In an elegant study using single-cell RT-PCR combined with electrophysiological recording from neuronal patches acutely isolated from young rats, Geiger et al. (1995) have shown that AMPA receptor subunit composition expressed in various neuron types determines the kinetics and Ca^{2+}-permeability of the response. Hippocampal principal neurons desensitize relatively slowly in response to glutamate ($\tau_{desens.}$ around 10–16 ms) and have a low permeability to Ca^{2+}, while GABAergic interneurons show fast desensitization ($\tau_{desens.}$ around 5–6 ms) and high permeability to Ca^{2+}. These marked functional differences were ascribed to the abundant expression of flip versions of GluR1 and GluR2 in principal neurons, while interneurons expressed mainly flop-GluR4, with only low levels of GluR2. Western blot analysis revealed that the AMPA receptors on cultured hippocampal neurons consist of GluR1 and GluR2/3 subunits (though the presence of GluR4 was not investigated) (Hall and Soderling, 1997). While our cultures would be expected to contain both principal and GABAergic interneurons (Jensen et al., unpublished observations), our results for τ_{decay} for the three agonists (8, 12 and 17 ms for AMPA, Tri-F-AMPA and ACPA, respectively) straddle those reported by Geiger et al. (1995) for the action of glutamate on principal neurons and by Partin et al. (1996) for the action of AMPA on GluR1 expressed in human embryonic kidney cells. Moreover, all five cultured hippocampal neurons tested here showed a linear *I–V* relationship in the presence of AMPA (Fig. 3). This circumstantial evidence makes it likely that GluR2 is present in the AMPA receptor complex expressed in hippocampal neurons, which has been confirmed by recent RT-PCR experiments performed in our laboratory (Dai et al., in preparation).

The observed rank order of potency for plateau responses is in full agreement with that obtained using extracellular recordings in rat cortical slices (Madsen et al., 1992) and in [^{3}H]AMPA binding displacement studies (Madsen and Wong, 1992; Nielsen et al., 1998). The time-scales of these experiments are likely to reflect steady-state conditions for the action of AMPA.

4.2. Maximum responses to the AMPA analogues

The relative maximum response was determined for steady-state plateau responses evoked by near-saturating concentrations of the agonists and normalized to the response evoked by a saturating concentration of AMPA. The maximum response evoked by Tri-F-AMPA was 60% greater than that to AMPA, while the maximum response evoked by ACPA was twice the size (Fig. 2B). Tri-F-AMPA and AMPA would, therefore, appear to be partial agonists with respect to ACPA. As to whether ACPA itself is a full agonist is still unknown. It should be noted that studies following blockade of receptor desensitization may well have yielded different maximum responses to the agonists. From a functional point of view, however, the relative degree of agonism should be assessed with intact receptor modulatory mechanisms.

4.3. Response kinetics

In outside-out patches stimulated with 1 mM AMPA, the peak currents ranged from a few pA to ~1800 pA. It is likely that patches exhibiting very large currents had been isolated from membrane areas of synaptic contacts or 'hot-spots'.

The agonist-evoked peak current is a convenient parameter to quantify responses. The peak represents the instant when recruitment of new ionophores to conducting states is exactly balanced by the passage of others into the desensitized state (Patneau et al., 1993). Changes in either of these factors would be expected to alter the amplitude and timing of the peak. The largest peak response is attained when receptors are activated as rapidly and synchronously as possible, as is achieved with near-instantaneous application of the agonist ('concentration jump'). The shape of the response is then determined by the affinity of the receptors, along with the kinetics of association, activation, desensitization, dissociation and deactivation. Since there was no significant difference in the 10–90% rise-time or the times-to-peak of the responses to the three agonists (Fig. 5, Table 2), the *absolute* rate-of-rise will vary in accordance with the amplitudes of the responses. In the absence of run-down, peak responses to ACPA and Tri-F-AMPA would be larger than to AMPA, and have correspondingly larger rates-of-rise. This would infer that these agonists have correspondingly greater association and/or transduction rates with AMPA receptors. To the best of our knowledge, association rate constants have not been studied for non-NMDA agonists. However, the kinetic constants for a range of NMDA receptor antagonists differ markedly (Benveniste and Mayer, 1991), so an equivalent situation for AMPA receptor agonists might be expected to prevail.

The decay of responses in the maintained presence of agonist reflects desensitization of the receptors. During the plateau phase, there is an equilibrium between ionophores passing between the open and desensitized (occupied, but closed) states. The larger plateau responses to Tri-F-AMPA and ACPA means that receptors desensitize to a lesser extent than in the presence of AMPA. There was, however, a strong correlation between the size of the plateau response from whole-cell recordings and τ_{decay} from outside-out patches ($r = 0.96$, Fig. 6). This suggests that the speed and extent of the decay are inextricably linked.

That ligands which interact with non-NMDA receptors can have different effects on desensitization is illustrated by the actions of competitive non-NMDA receptor antagonists, which also reduce desensitization to a greater or lesser extent. Potent antagonists such as the quinoxalinediones reduce desensitization and have less efficacious actions on the plateau compared to peak responses (Parsons et al., 1994). Cyclothiazide, on the other hand, causes very marked reduction of desensitization of AMPA receptors, but nevertheless acts as a weak receptor antagonist (Patneau et al., 1993; Paternain et al., 1996). Between these two extremes are a group of low potency receptor antagonists which reduce responses evoked by low concentrations of agonist, but actually enhance responses at high concentrations of agonist by reducing receptor desensitization. 2-Amino-3[3-(carboxymethoxy)-5-methylisoxazol-4-yl]propionate (AMOA) is an example of such a compound (Wahl et al., 1992), while similar properties have been described for Evans Blue (Schürmann et al., 1997). Since a competitive antagonist behaves as an agonist with low or zero intrinsic activity it is perhaps not surprising that agonists can activate desensitization to varying extent. Indeed, kainate is a prominent example of an agonist which acts on expressed AMPA receptors with little or no desensitization (Sommer et al., 1990).

Wahl et al. (1996) have discussed the possibility that the distal acid group in AMPA and its analogues plays a major role in determining the degree of receptor activation in the desensitised state (pK_A values are 2.2 for ACPA, 3.4 for Tri-F-AMPA and 4.8 for AMPA (Madsen et al., 1986; Madsen et al., 1992; Wahl et al., 1996)). It is possible that the distal acid group could control the rate of desensitization, which slows as it becomes more acid.

5. Concluding remarks

In relation to ACPA, both Tri-F-AMPA and AMPA appear to be partial agonists at the AMPA receptors expressed in cultured hippocampal neurons. The desensitization rate constants of responses evoked by ACPA and Tri-F-AMPA are longer than for AMPA. This means that a greater fraction of the ionophores are conducting under steady-state conditions, which gives rise to correspondingly larger plateau responses. The slowing of desensitization will also give rise to a larger peak response, though an increase in association and/or transduction rate constants for ACPA and Tri-F-AMPA may also be involved.

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