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Short communication

Differential modulation of the GYKI 53784-induced inhibition of AMPA currents by various AMPA-positive modulators in cerebellar Purkinje cells

Gábor Kapus [a,*], Ilona Bódi [a,1], Ágnes Pataki [a], Jean-Patrick Gueritaud [b], József I. Székely [a], István Tarnawa [a,2]

[a] *Institute for Drug Research, P.O. Box 82, H-1325 Budapest, Hungary*
[b] *Unite de Neurocybernetique Cellulaire, CNRS UPR 9041, 280, Bd. Sainte Marguerite, 13009 Marseille, France*

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Abstract

The effects of various (*S*)-α-amino-3-hydroxy-5-methyl-4-izoxazole-propionate (AMPA) receptor modulators on AMPA-induced whole-cell currents were compared in isolated rat cerebellar Purkinje cells. The positive modulators, aniracetam, cyclothiazide, 1-(1,3-benzodioxol-5-ylcarbonyl)-piperidine (1-BCP), and 1-(quinoxaline-6-ylcarbonyl)-piperidine (BDP-12), dose-dependently potentiated the steady-state component of AMPA currents. The negative modulator, (−)1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-4,5-dihydro-3-methylcarbamoyl-2,3-benzodiazepine (GYKI 53784), dose-dependently suppressed AMPA responses. Its concentration–response curve was shifted to the right in a parallel fashion by all positive modulators, indicating a competitive type of interaction. However, the relative potencies of the positive modulators were different with regard to the enhancement of AMPA responses and the reversal of GYKI 53784-induced inhibition, respectively. It is supposed that positive modulators act at multiple allosteric sites and that they interact with GYKI 53784 at only one of these sites.

Keywords: AMPA receptor; Purkinje cell; GYKI 53784; Aniracetam; Cyclothiazide; 1-BCP; BDP-12

1. Introduction

Recently, a considerable body of data has accumulated on allosteric modulation of (*S*)-α-amino-3-hydroxy-5-methyl-4-izoxazole-propionate (AMPA) receptors. Several chemically diverse groups of pharmaca, including aniracetam, cyclothiazide, 1-(1,3-benzodioxol-5-ylcarbonyl)-piperidine (1-BCP), 1-(quinoxaline-6-ylcarbonyl)-piperidine (BDP-12) and some other agents, have been found to allosterically potentiate AMPA currents (Bleakman and Lodge, 1998). In contrast, GYKI 52466, a 2,3-benzodiazepine, and some of its congeners, allosterically and selectively inhibit AMPA responses (Donevan and Rogawski, 1993; Paternain et al., 1995; Zorumski et al., 1993; Tarnawa and Vizi, 1998). Some early data suggested that the positive and negative modulators of AMPA receptors bind to a common allosteric site (Chapman et al., 1993; Palmer and Lodge, 1993; Zorumski et al., 1993). However, other studies indicated that the interaction between cyclothiazide and GYKI 52466 is of non-competitive nature, and that, therefore, they cannot bind to a common modulatory site (Johansen et al., 1995; Yamada and Turetsky, 1996; Rammes et al., 1996, 1998; Donevan and Rogawski, 1998). In addition, several recent findings indicate that even the chemically distinct subgroups of AMPA-positive modulators may have divergent mechanisms of action (Arai et al., 1996). As an extension of previous studies, in this study, two issues were addressed. Is it possible that positive and negative modulators of AMPA receptors induce their opposite actions through a common binding site? Do the different types of AMPA-positive modulators interact with a 2,3-benzodiazepine congener ((−)1-(4-aminophenyl)-4-methyl-7,8-methylen-

* Corresponding author. EGIS Pharmaceuticals Ltd., CNS Pharmacology, P.O. Box 100, H-1475 Budapest, Hungary. Tel.: +36-1-404-1000; fax: +36-1-407-4888.
E-mail address: sipka@freemail.hu (G. Kapus).
[1] Present address: College of Medicine Institute of Molecular Pharmacology and Biophysics, University of Cincinnati Medical Center, P.O. Box 670828, Cincinnati OH 45267-0828, USA.
[2] Present address: Gedeon Richter Chemical Works Ltd., P.O. Box 27, H-1475 Budapest, Hungary.

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edioxy-4,5-dihydro-3-methylcarbamoyl-2,3-benzodiazepine (GYKI 53784), also known as LY303070) differently or not?

2. Materials and methods

Purkinje cell cultures were obtained from cerebella of P6–8 Sprague–Dawley rats as described by Bleakman et al. (1996). The cells were plated on glass coverslips coated with poly-L-lysine and incubated at 37°C. The agonist-induced whole-cell currents were recorded with patch pipettes (3–8 MΩ). The internal solution contained (in mM) 140 KCl, 1 $MgCl_2$, 10 HEPES, 0.1 EGTA (pH 7.2). Currents were recorded using the Axopatch 1D patch clamp amplifier (Axon Instruments) at −70 mV. The signals were low-pass filtered at 2 kHz, digitised at 10 kHz (Digidata 1200, Axon) and stored on an IBM PC. Data were analysed by using pClamp 6.0 software (Axon). The cells were continuously perfused at a rate of 0.5 ml/min with external recording solution containing (in mM) 138 NaCl, 5 $CaCl_2$, 1 $MgCl_2$, 5 KCl, 10 HEPES, 10 glucose, (pH 7.35). Drugs were administered through a multibarrel, gravity-driven drug delivery system controlled by electromagnetic valves. GYKI 53784, aniracetam, 1-BCP and BDP-12 were synthesised at the Institute for Drug Research, Hungary. AMPA and cyclothiazide were obtained from Tocris and RBI, respectively, while all the other chemicals were from Sigma. Modulators were dissolved in dimethyl sulphoxide (DMSO) (10 mM) and further diluted in saline prior to use. In its final concentration, DMSO per se did not induce noticeable alterations in the current responses. Means ± S.E.M. were calculated from data collected from 4 to 9 cells. IC_{50} values and the slopes of dose–response curves were computed using sigmoidal curve fitting to a logistic equation (Origin 4.1). The statistical significance of the potentiation by the positive modulators of AMPA-induced responses was assessed by paired Student's *t*-test. In the drug interaction studies, the various treatment groups were compared by one-way analysis of

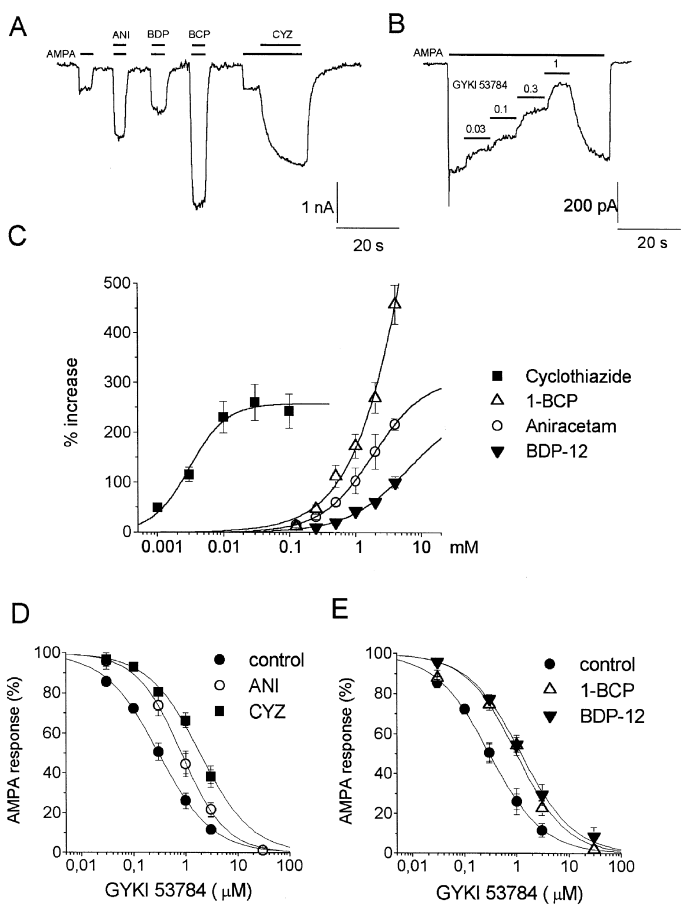

Fig. 1. Effect of positive and negative allosteric modulators on the steady-state component of AMPA-induced inward currents in isolated rat cerebellar Purkinje cells. (A) A representative trace of whole-cell currents induced by 5 μM of AMPA. Note the potentiation of AMPA responses by 1-BCP (BCP) or BDP-12 (BDP) or aniracetam (ANI) (all at 2 mM) or 30 μM of cyclothiazide (CYZ). (B) Concentration-dependent inhibition of the AMPA (5 μM) current by GYKI 53784. (C) Dose dependence of the potentiation of AMPA (5 μM) responses by different positive modulators. (D and E) Parallel rightward shift of the dose–response curve of GYKI 53784 for inhibition of the AMPA (5 μM) elicited steady-state current by co-application of CYZ (30 μM) or ANI (4 mM) (panel D) or 4 mM of 1-BCP or BDP-12 (panel E). On panels C, D, E, symbols and vertical bars represent the means ± S.E.M. of 5–9 independent experiments. The curves on panels C, D and E were calculated by fitting the data to a logistic equation.

variance (ANOVA) followed by Duncan's assay for multiple paired comparisons.

3. Results

Administration of AMPA (5 μM) to freshly isolated cerebellar Purkinje cells induced reproducible whole-cell inward current responses, which usually consisted of a rapidly declining peak and a stable plateau component (423 ± 28 pA, $n = 82$) (Fig. 1A,B). Since a gravity-driven slow perfusion technique was used, approximately 150–200 ms elapsed between the start of infusion and the full development of steady-state AMPA responses. Thus, the peak amplitudes could not be measured reliably. Therefore, only the steady-state component of the current responses was considered. In our experiments, 5 μM AMPA elicited nearly maximal steady-state current responses.

None of the positive modulators elicited any current per se; however, they robustly potentiated the steady-state AMPA responses (Fig. 1A). The estimated EC_{50} values for cyclothiazide, aniracetam, 1-BCP and BDP-12 were 3.05 μM, 1.86, 11.71 mM and 6.3 mM, respectively (Fig. 1C). Because of their limited water solubility, the plateau concentration could only be reached by cyclothiazide, which casts some doubt on the validity of these calculated values. Based on the EC_{50} values, the rank order of potency was:

cyclothiazide ≫ aniracetam > BDP-12 > 1-BCP.

However, with regard to the maximal degree of potentiation, at the highest drug concentrations used, the rank order was (Fig. 1C):

1-BCP > cyclothiazide > aniracetam > BDP-12.

As shown in Fig. 1B, GYKI 53784 depressed the steady-state component of AMPA (5 μM)-induced currents in a concentration-dependent fashion ($IC_{50} = 0.32 \pm 0.04$ μM). All four AMPA-positive modulators shifted the concentration–response curve of GYKI 53784 to the right. The IC_{50} of GYKI 53784 was multiplied by 5.90, 2.63, 2.90 and 3.94 in the presence of cyclothiazide (30 μM), aniracetam (4 mM), 1-BCP (4 mM) and BDP-12 (4 mM), respectively (Table 1). In spite of the robust increases in the IC_{50} value of GYKI 53784, the slope of the concentration–inhibition curve did not change in the presence of any positive modulator, i.e. the rightward shift was apparently parallel (Fig. 1D,E; Table 1). With regard to the reversal of GYKI 53784-elicited inhibition, the rank order of potency was:

cyclothiazide > BDP-12 > 1-BCP > aniracetam.

For a better overview of the experimental data, we have used the operative terms potency ratio (PR) and dose ratio (DR), respectively (see Table 1). PR indicates how many times the steady-state component of the AMPA current was increased in the presence of positive modulators, whereas DR is the ratio of inhibitory IC_{50} values for GYKI 53784 in the presence and absence of the positive modulators, respectively. Finally, relative efficacy (RE) is the ratio PR/DR. Comparison of the PR and DR values revealed that the relative efficacy of the AMPA-positive modulators was different with regard to potentiation of the AMPA current and to the suppression of GYKI 53784-induced inhibition, respectively (Table 1). Comparison of the relative efficacy of AMPAkines at 4 mM suggested that 1-BCP was more effective in potentiation of the AMPA responses (RE = 0.52), whereas BDP-12 was more potent in reversal of the GYKI 53784-induced inhibition of AMPA currents (RE = 1.96) (Fig. 1E; Table 1). The RE value of aniracetam (0.83 at 4 mM) was between that of 1-BCP and that of BDP-12. In the case of cyclothiazide, the RE value varied with its concentration (0.71 and 1.64 at 3 and 30 μM, respectively) (Table 1).

Table 1
Potentiation of the steady-state component of the AMPA-induced current and reversal of the GYKI 53784-induced inhibition by positive modulators. Values are the means ± S.E.M. of 4–9 experiments

AMPA positive modulator	PR[a]	IC_{50} (μM) GYKI 53784	DR[b]	Hill coefficient	RE = DR/PR
No (control)	1	0.32 ± 0.04	1	0.84 ± 0.04	1
3 μM cyclothiazide	2.16[c] ± 0.14	0.49[d] ± 0.05	1.54	0.99 ± 0.11	0.71
30 μM cyclothiazide	3.60[c] ± 0.36	1.87[e] ± 0.11	5.90	0.84 ± 0.04	1.64
500 μM aniracetam	1.61[c] ± 0.30	0.27 ± 0.03	0.85	1.05 ± 0.11	0.53
4 mM aniracetam	3.17[c] ± 0.12	0.83[d] ± 0.09	2.63	1.04 ± 0.20	0.83
500 μM 1-BCP	2.13[c] ± 0.22	0.48[d] ± 0.05	1.50	0.94 ± 0.15	0.71
4 mM 1-BCP	5.58[c] ± 0.39	0.92[e] ± 0.13	2.90	0.90 ± 0.12	0.52
4 mM BDP-12	2.01[c] ± 0.11	1.26[e] ± 0.12	3.94	0.88 ± 0.12	1.96

[a] ~ fold increase of AMPA response.
[b] ~ fold increase of IC_{50} of GYKI 53784.
[c] $P < 0.01$ comparing groups treated with AMPA (5 μM) alone and AMPA plus a positive modulator (Student's *t*-test).
[d] $P < 0.05$ comparing groups treated with AMPA (5 μM) plus one of the increasing concentrations of GYKI 53784 in the presence and absence of a positive modulator by ANOVA followed by Duncan's assay.
[e] $P < 0.01$ comparing groups treated with AMPA (5 μM) plus one of the increasing concentrations of GYKI 53784 in the presence and absence of a positive modulator by ANOVA followed by Duncan's assay.

4. Discussion

As seen above, GYKI 53784 reduced the steady-state component of AMPA responses in a concentration-dependent fashion. All three types of AMPA-positive modulators, i.e. cyclothiazide, aniracetam and the piperidine derivatives (1-BCP and BDP-12), were inactive per se but they dose-dependently potentiated AMPA-induced inward currents. Furthermore, all these modulators shifted the AMPA inhibitory dose response curve of GYKI 53784 to the right in a parallel fashion. These findings are compatible with the existence of a common binding site at which positive and negative modulators of AMPA receptors interact with each other competitively (Zorumski et al., 1993; Palmer and Lodge, 1993). However, a closer look at our own data and those in the literature indicates a more complex regulation of AMPA channel function. If the AMPA-positive and -negative modulators interact competitively at a common binding site, then their relative potencies to enhance AMPA responses (PR) and to reverse the inhibitory action of GYKI 53784 (DR) should be correlated. However, this was not the case in our study. For example, 1-BCP was a strong potentiator of AMPA currents, but it was a relatively weak antagonist of the effect of GYKI 53784, while the opposite was seen with BDP-12. Thus, the RE values (i.e. the PR/DR ratio) varied with the compound examined, and in the case of cyclothiazide, even with its dose (Table 1). Although the RE values were not determined in exactly equiactive concentrations of the positive modulators, the great differences in RE values indicate that distinct binding sites may be involved in the potentiation of the AMPA response and the reversal of GYKI 53784-induced inhibition. Several earlier data are in line with this assumption. In the studies of Rammes et al. (1996, 1998), two congeners of GYKI 53784 (GYKI 52466 and GYKI 53405) did not show true competition with regard to the onset and offset of AMPA currents. Furthermore, Yamada and Turetsky (1996) concluded from their electrophysiological experiments that GYKI 52466 could not completely displace cyclothiazide from its binding site. In addition, working with recombinant AMPA receptors, Partin and Mayer (1996) found that certain mutations of the flip/flop domain significantly changed the sensitivity of the AMPA receptors to cyclothiazide, while leaving the efficacy of the GYKI compound unaltered.

The overall picture is further complicated by the observation that the mechanisms of potentiation of early and late AMPA current components are different. While 2,3-benzodiazepines equally inhibit the peak and steady-state components of AMPA currents (Rammes et al., 1996), i.e. they have no effect on receptor desensitisation, various AMPA-positive modulators preferentially facilitate (with different kinetics) the early and late phases, respectively (Arai et al., 1996; Rammes et al., 1998). However, in our study, the block of steady-state AMPA currents by 2,3-benzodiazepines seemed to be competitively antagonised by cyclothiazide, as reported by others (Johansen et al., 1995; Rammes et al., 1996, 1998; Donevan and Rogawski, 1998). These apparent contradictions in the literature may be resolved by presuming the existence of multiple allosteric modulatory sites. We propose that some effects of positive modulators, such as the enhancement of the plateau current amplitude, are mediated by the putative 2,3-benzodiazepine site. Nevertheless, another class of binding sites may be involved in AMPA receptor desensitisation, which is insensitive to 2,3-benzodiazepines (Rammes et al., 1996; Partin and Mayer, 1996). In conclusion, our study provides further experimental data indicating that the mechanism of action of AMPA-positive modulators is not uniform. They may act differently on the 2,3-benzodiazepine-sensitive site and on one or more 2,3-benzodiazepine-insensitive allosteric modulatory sites.

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