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Short communication

Differential effects of five glycine site antagonists on NMDA receptor desensitisation

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Abstract

The effects of five glycine site antagonists were comparatively examined on maximal and plateau currents evoked by 200 μM *N*-methyl-D-aspartate (NMDA) in the presence of 1 μM glycine in cultured cerebrocortical cells of the rat using whole-cell patch-clamp technique. 5,7-Dichlorokynurenic acid, ACEA-1021 (5-nitro-6,7-dichloro-quinoxalinedione), L-695,902 (methyl 7-chloro-4-hydroxy 2(1*H*)-quinolone-3-carboxylate), LY-294,619 (5,7-dichloro-3-(4-hydroxphenyl)-4-hydroxyquinolin-2(1*H*)-one) and RPR-104,632 (2-(3-bromo-benzyl)-6,8-dichloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-3-carboxylic acid) caused concentration-dependent inhibition of NMDA-activated currents. However, antagonists showed different relative efficacies to block peak currents and plateau currents, characterised by the following IC_{50} ratios: L-695,902: 0.98; RPR-104,632: 1.06; ACEA-1021: 1.69; LY-294,619: 1.71; and 5,7-dichlorokynurenic acid: 3.42. Our findings indicate a heterogeneity of glycine site antagonists in affecting NMDA receptor desensitisation, and suggest potential differences in their pharmacologies.

Keywords: NMDA receptor; Glycine coagonist site; Desensitization; 5,7-Dichlorokynurenic acid; ACEA-1021; L-695,902; LY-294,619; RPR-104,632

1. Introduction

The glycine-sensitive coagonist site of the NMDA receptor complex is generally regarded as a favourable target for NMDA receptor antagonists (Leeson and Iversen, 1994). At doses evoking remarkable anticonvulsant and neuroprotective effects glycine site antagonists exhibit less severe motoric and cognitive side effects than NMDA-competitive antagonists and are free of phencyclidine-like psychotomimetic actions, a characteristic for NMDA receptor channel blockers (see Leeson and Iversen, 1994).

Desensitisation of the NMDA receptor complex is mediated by glycine sensitive and insensitive (Vyklicky, 1993) mechanisms. As endogenous coagonist, glycine potentiates cation currents evoked by NMDA (Johnson and Asher, 1987) via inhibition of desensitisation of NMDA receptors (Mayer et al., 1989). Antagonists of the glycine site inhibit NMDA-induced currents by unmasking the effect of glycine on the desensitisation of NMDA receptors (Parsons et al., 1993), although L-695,902 (methyl 7-chloro-4-hydroxy 2(1*H*)-quinolone-3-carboxylate), a full antagonist at the glycine site, has been reported to decrease NMDA-induced currents without any effect on desensitisation (Grimwood et al., 1993).

In the present study, we provide evidence that glycine site antagonists are heterogenous in blocking maximal and plateau currents activated by NMDA in cultured cerebrocortical neurons of the rat, suggesting that the coagonist effect of glycine may involve two separable components with potentially distinct pharmacologies. The following glycine site antagonists were selected for the present study: 5,7-dichlorokynurenic acid (Foster et al., 1992), ACEA-1021 (5-nitro-6,7-dichloro-quinoxalinedione) (Woodward et al., 1995), L-695,902 (Grimwood et al., 1993), LY-294,619 (5,7-dichloro-3-(4-hydroxphenyl)-4-hydroxy-quinolin-2(1*H*)-one) (McQuaid et al., 1993), RPR-104,632 (2-(3-bromo-benzyl)-6,8-dichloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-3-carboxylic acid) (Jimonet et al., 1994).

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2. Materials and methods

2.1. Animals and tissue cultures

Primary cultures were prepared from foetal rat (Sprague-Dawley, Charles River, Budapest) cerebral cortex on day 17 of pregnancy as described in detail earlier (Erdõ et al., 1990; Lakics et al., 1995). Briefly, cortical tissue was isolated, trypsinised, and the cells plated at a density of 3×10^5 per well onto coverslips coated with poly-D-lysine, in a volume of 0.5 ml. Cultures were suspended in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% foetal calf serum and maintained at 37°C in a humidified 5% CO_2 atmosphere for up to 14 days.

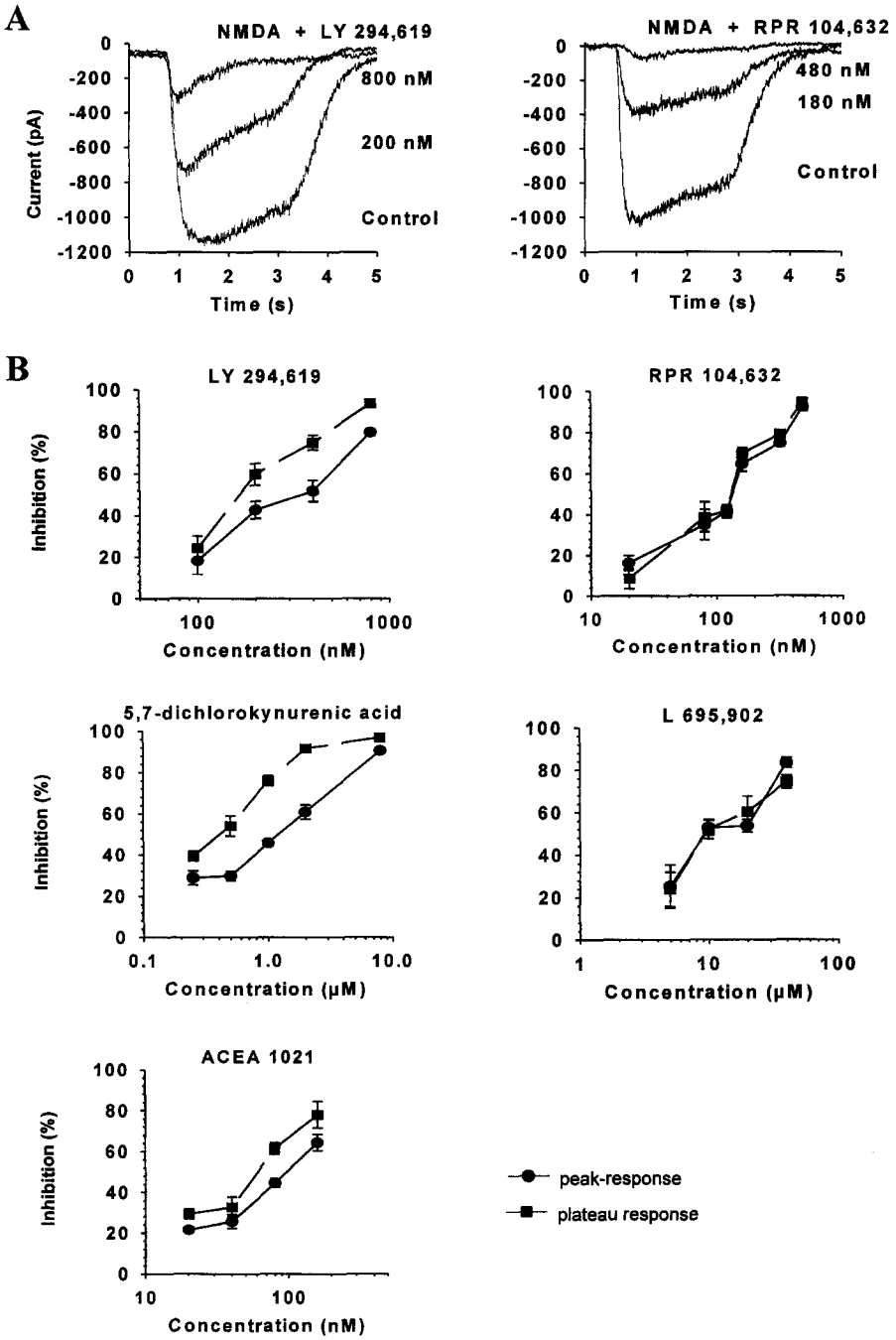

Fig. 1. Inhibition of cation currents activated by 200 μM NMDA in the presence of 1 μM glycine in cultured cerebrocortical neurones. (A) Representative recordings illustrating the equal potency of RPR-104,632 to block maximal current and plateau current, and the more pronounced potency of LY-294,619 to inhibit plateau response than peak response. (B) Concentration-response curves for inhibition of peak currents (■) and plateau currents (●) by various glycine site antagonists. Note the variable efficacies of antagonists in blocking the two types of current. For IC_{50} values, see Table 1. DCKA = 5,7-dichlorokynurenic acid. (A) Representative recordings. Repeated experiments gave similar results. (B) Points and vertical bars represent mean ± S.E.M. ($n = 3$–5).

2.2. Patch clamp experiments

Whole-cell patch clamp experiments were performed at room temperature on the stage of an inverted phase-contrast microscope (Olympus, IMT2). Coverslips with the cultured cells (7–14 days in culture) were transferred into the recording chamber, rinsed and continuously perfused by gravity with an extracellular solution containing (in mM): NaCl 140, KCl 5, $CaCl_2$ 0.2, glucose 20, sucrose 10, Hepes 10, tetrodotoxin 0.0003, glycine 0.001. Patch pipettes (5–10 MΩ) were prepared from filament containing standard-wall borosilicate glass capillaries (outer diameter 1.2 mm, Clark Electromedicals) with a micropipette puller (P-87, Sutter). The intracellular solution contained (in mM) CsCl 110, NaCl 15, $CaCl_2$ 0.1, $MgCl_2$ 2, ATP 2, EGTA 1, tetraethylammonium-Cl 10, Hepes 10.

Whole-cell currents were recorded with an Axopatch 200 A amplifier using the pClamp 5.5 software (AXON). Capacitive transients and series resistance were compensated. Signals were filtered at 1 kHz and sampled at 1 kHz. Drugs were dissolved in the extracellular solution and were administered directly to the cells with a seven-barrelled gravity driven fast drug administration system (Maksay et al., 1994). To evoke currents 2 s pulses of 200 μM NMDA were applied in every 30 s. This concentration of NMDA evokes a current which is about 70% of the maximal response. Higher concentrations of NMDA increased the Ca^{2+}-dependent component of desensitisation.

2.3. Data analysis

The amplitudes of NMDA-evoked currents were measured using the pClamp 5.5 program. Inhibition was calculated on the basis of amplitudes of maximal responses and plateau responses reached 2 s after NMDA application, in the presence and absence of antagonists (Parsons et al., 1993). IC_{50} values were calculated by direct curve fitting (SigmaPlot for Windows). The effect of each antagonist at each concentration was measured on 3–5 separate cells. Results were expressed as mean ± S.E.M.

2.4. Drugs

5,7-Dichlorokynurenic acid was obtained from Tocris, ACEA-1021, L-695,902, LY-294,619, RPR-104,632 were synthesised in our chemistry laboratories. The identity and purity (> 97%) of each antagonist was analysed by nuclear magnetic resonance spectroscopy. All other chemicals were obtained from Sigma and were of analytical purity.

3. Results

Cation currents evoked in cultured cortical neurones by 200 μM NMDA in the presence of 1 μM glycine were inhibited by each glycine site antagonist in a concentration-dependent manner (Fig. 1). The potencies of various antagonists to block maximal and plateau responses to NMDA were variable. Typically, certain compounds, e.g. RPR-104,632, inhibited both responses to the same extent, whereas others, such as LY-294,616, more potently blocked plateau currents than peak responses (Fig. 1A). The IC_{50} values estimated for the inhibition of peak and plateau responses and their ratios varied between 0.98 and 3.42 (Table 1).

Table 1
IC_{50} values of glycine site antagonists to block 200 μM NMDA-induced peak and plateau currents in cultured neurones of rat cerebral cortex in the presence of 1 μM glicine

Antagonist	IC_{50} (nM)		
	Peak current	Plateau current	Ratio
5,7-Dichlorokynurenic acid	1006 ± 160	294 ± 60	3.42
LY-294,619	307 ± 39	179 ± 16	1.71
ACEA-1021	95.6 ± 10.5	56.7 ± 8.2	1.69
RPR-104,632	123 ± 15	116 ± 12	1.06
L-695,902	12100 ± 2500	12300 ± 1900	0.98

Values are mean ± S.D., $n = 4–5$.

4. Discussion

The present findings provide evidence for a heterogeneity of glycine site antagonists in terms of potency to inhibit NMDA-evoked peak currents and to unmask the glycine inhibition of desensitisation of NMDA receptor currents. This phenomenon suggests that the binding to the glycine site and the enhancement of desensitisation by antagonists may have different structure requirements. Moreover, this heterogeneity of glycine site antagonists in terms of influencing desensitisation may contribute to differences in pharmacological profiles, a recently recognised feature of certain glycine site antagonists (Kehne et al., 1995).

Antagonists that increase desensitisation at concentrations lower than those needed to inhibit peak currents, e.g. 5,7-dichlorokynurenic acid, LY-294,616 and ACEA-1021, are expected to possess less severe side effects than those exerting both effects at the same concentration. However, this hypothesis needs to be justified in in vivo experiments. Unfortunately, the compound showing the highest ratio of potencies, i.e. 5,7-dichlorokynurenic acid, does not cross the blood-brain barrier. Therefore, further studies are needed to find systemically active glycine site antagonists with several-fold higher potency to increase desensitisation than to block peak currents. Studies attempting to justify the pharmacological significance of the above-described heterogeneity in terms of therapeutic index and side-effect profile are under progress.

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