Neuropharmacology 57 (2009) 242–249

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Electrophysiological characterisation of the actions of kynurenic acid at ligand-gated ion channels

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ARTICLE INFO

Article history:
Received 10 December 2008
Received in revised form
22 May 2009
Accepted 1 June 2009

Keywords:
Kynurenic acid
NMDA receptor
α7 Nicotinic receptor
GABA-A receptor
Glycine receptor
DMSO

ABSTRACT

To better understand the effects of the tryptophan metabolite kynurenic acid (kynA) in the brain, we characterised its actions at five ligand-gated ion channels: NMDA, AMPA, $GABA_A$, glycine and α7 nicotinic acetylcholine receptors. Using whole-cell patch-clamp recordings, we found that kynA was a more potent antagonist at human NR1a/NR2A compared with NR1a/NR2B receptors (IC_{50}: 158 μM and 681 μM, respectively; in 30 μM glycine). KynA inhibited AMPA-evoked currents to a similar degree in cultured hippocampal neurons and a human GluR2(flip/unedited) cell line (IC_{50}: 433 and 596 μM, respectively) and at higher concentrations, kynA also inhibited the strychnine-sensitive glycine receptor (~35% inhibition by 3 mM kynA). Interestingly, kynA inhibited the peak amplitude (IC_{50}: 2.9 mM for 10 μM GABA) and slowed the decay kinetics of GABA-evoked currents in cultured neurons. In contrast, we found that kynA (1–3 mM) had no effect on ACh-evoked, methyllycaconitine (MLA)-sensitive currents in a human α7 nicotinic receptor (nAChR) cell line, rat hippocampal neurons in primary culture or CA1 *stratum radiatum* interneurons in rat brain slices. However, DMSO (>1%) did inhibit α7 nAChR-mediated currents. In conclusion, kynA is an antagonist at NMDA, AMPA and glycine receptors and a modulator of $GABA_A$ receptors, but we find no evidence for any effect of kynA at the α7 nAChR.

1. Introduction

The kynurenine pathway of tryptophan metabolism processes the majority of dietary tryptophan and generates a number of neuroactive substances. The neuroprotective kynurenic acid (kynA) is predominantly synthesised by kynurenine aminotransferases in astrocytes and constitutes a 'dead-end' metabolite of the pathway (Schwarcz and Pellicciari, 2002). KynA has been demonstrated to inhibit glutamatergic transmission in a number of native systems, affecting NMDA-induced responses to a greater extent than quisqualate- or kainate-evoked signals (Perkins and Stone, 1982; Ganong et al., 1989; reviewed by Stone, 1993). At the NMDA receptor (NMDAR), kynA binds in a competitive manner to both the glutamate- and glycine binding sites, with a higher affinity for the latter (Birch et al., 1988; Bertolino et al., 1989; Parsons et al., 1997; Hilmas et al., 2001). KynA has also been reported to inhibit other ionotropic receptors, notably the AMPA receptor (AMPAR) and α7 nAChR (Hilmas et al., 2001; Prescott et al., 2006).

There is increasing attention surrounding the possible involvement of the kynurenine pathway in pathological conditions such as schizophrenia, Huntington's disease and CNS infections (reviewed by Stone and Darlington, 2002; Schwarcz and Pellicciari, 2002). A number of studies have documented the range of effects kynA has in behavioural models (Erhardt et al., 2004; Chess et al., 2007) and on dopaminergic, cholinergic and glutamatergic transmission (Carpenedo et al., 2001; Erhardt et al., 2001b; Shepard et al., 2003; Rassoulpour et al., 2005). To better understand how kynA may mediate these effects, it is important to quantify its actions at the receptor level and to address certain discrepancies in the literature. In light of this, we conducted a study to rigorously characterise the effects of kynA on the NMDAR, AMPAR, $GABA_A$ receptor ($GABA_AR$), glycine receptor (GlyR) and α7 nAChR using recombinant cell lines, transient transfections in HEK293 cells, cultured neurons and acute brain slices.

2. Materials and methods

2.1. Transient transfections and cell lines

HEK293-MSRII cells were plated onto poly-D-lysine(PDL)-coated coverslips (30–40K cells/well) for 24 h and then transfected with EGFP and NR1a + NR2A or NR1a + NR2B plasmids using Fugene6 (ratio 1:1:5; Roche Applied Sciences). Human and rat orthologues of NR1a, NR2A and NR2B were tested. Cells were then kept in media at 37 °C, containing the NMDAR antagonist D-amino-phosphonovalerate (D-AP5; 100 μM), and were used 24–48 h post-transfection. The

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doi:10.1016/j.neuropharm.2009.06.003

GluR2(flip/unedited)-HEK stable cell line was split twice weekly and plated on PDL-coated coverslips 24 h before experimentation. The GH4C1 cell line stably expressing human α7 nAChR (α7-GH4; Virginio et al., 2002) was also plated onto PDL-coated glass coverslips, kept at 30 °C and used 1–3 days after plating.

2.2. Rat hippocampal neurons in primary culture

Neuronal cultures were prepared from embryonic rat brains harvested following overdose by CO_2 inhalation in accordance with GlaxoSmithKline animal welfare guidelines and the UK Animals (Scientific Procedures) Act 1986. The dissected hippocampi were placed into an ice-cold medium: Hank's balanced salt solution (HBSS; Ca^{2+}- and Mg^{2+}-free); pyruvate, 1 mM; penicillin/streptomycin, 100 U/ml; Hepes, 10 mM; $NaHCO_3$, 0.035%. Trypsin/EDTA was diluted in HBSS with sodium pyruvate (Ca^{2+}- and Mg^{2+}-free) and the tissue was trypsinized for 30 min at 37 °C. Tissue pieces were physically dissociated and neurons were plated onto PDL-coated coverslips in the following plating medium: neurobasal medium + 1 mM sodium pyruvate; penicillin/streptomycin, 100 U/ml; B27 supplement 1× L-glutamine, 1 mM. Half of the volume of medium was replaced twice weekly and the cells were used for recordings from 7 to 18 days *in vitro*.

2.3. Hippocampal slice preparation

Sprague–Dawley rats (postnatal day (P)12–24) were deeply anaesthetized with isoflurane by inhalation and the brains were removed following decapitation in accordance with GlaxoSmithKline animal welfare guidelines and the UK Animals (Scientific Procedures) Act 1986. Using a vibratome (HM650V, Carl Zeiss Ltd), horizontal hippocampal slices (300 μm thick) were cut in ice-cold artificial cerebrospinal fluid (aCSF) solution of the following composition (mM): NaCl, 125; KCl, 2.5; $NaHCO_3$, 26; $NaH_2PO_4.H_2O$, 1.25; glucose, 25; $CaCl_2$, 1, $MgCl_2$, 2, bubbled with 95% O_2/5% CO_2. Slices were incubated at room temperature in aCSF for an hour before experimentation and used for up to 8 h later.

2.4. Whole-cell patch-clamp recordings

Cultured hippocampal neurons or HEK293 cells were perfused with an external solution containing (mM): NaCl, 145; KCl, 2.5; HEPES, 10; glucose, 10; $CaCl_2$, 1.5; $MgCl_2$, 1; pH 7.4 with NaOH. When recording from cultured neurons, tetrodotoxin (TTX; 0.5 μM) was perfused throughout the recordings to block action potential firing. In recordings of NMDA-evoked currents, $MgCl_2$ was omitted from the external solution. Patch pipettes were filled with (mM): $KMeSO_4$, 135; HEPES, 10; NaCl, 4; Mg-ATP, 4; Na_2GTP, 0.2; EGTA, 0.5; pH 7.3; 280–300 mOsmol/kg. In some experiments, amphotericin B (0.5–0.8 mg/ml) was added to the internal solution in order to record in the perforated-patch mode. Membrane currents were recorded using an Axopatch 200B amplifier and pClamp9 software (Molecular Devices) and the holding membrane potential was set at −60 or −70 mV for NMDAR, AMPAR and alpha7 nAChR recordings and at −20 mV for $GABA_AR$ and GlyR recordings. Solutions containing test compounds were applied via a dual-barrel fast perfusion system (RSC-160; Biologic).

Hippocampal slices were superfused (2–3 ml min^{-1}) at room temperature with aCSF (mM): NaCl, 125; KCl, 2.5; $NaHCO_3$, 26; $NaH_2PO_4.H_2O$, 1.25; glucose, 25; $CaCl_2$, 2; $MgCl_2$, 1; bubbled with 95% O_2/5% CO_2. Neurons in the CA1 *stratum radiatum* of acutely isolated brain slices were visualized under IR-DIC optics (Nikon). Membrane currents were recorded by whole-cell voltage-clamp (holding potential = −70 mV) using a Multipatch 700B amplifier and pClamp9 software (Molecular Devices). In pressure ejection experiments, a glass pipette of tip diameter 50–150 μm was positioned adjacent to the target cell body and was used to apply ACh by pressure micro-ejection (50–100 ms duration, 2 min interval; 5–15 p.s.i., Picospritzer, Warner Instruments) in the presence of TTX (0.5 μM), D-AP5 (50 μM), NBQX (2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo(f)quinoxaline-7-sulphonamide disodium; 5 μM) and picrotoxin (100 μM). For sIPSC recordings in slices (>P21; Mok and Kew, 2006), patch pipettes were filled with an internal solution containing a high concentration of chloride (mM): CsCl, 140; NaCl, 2; CsHEPES, 10; CsEGTA, 10; 300 mOsmol/kg. Patch pipettes had tip resistances of 4–8 MΩ when filled with internal solution and series resistance compensation was employed (typically, 70% at 1–1.3 kHz). To isolate sIPSCs pharmacologically, NBQX (5 μM), D-AP5 (50 μM), atropine (10 μM) and DHβE (dihydro-β-erythroidine; 10 μM) were perfused to block NMDA-, AMPA-, muscarinic and α4β2 nicotinic receptors, respectively.

Data analysis of agonist-evoked currents was performed using pClamp9 (Molecular Devices) and Origin7.5 (Original Lab Corp., Northampton, MA, USA) software. Concentration–response curves were fitted with the Hill equation:

$$y = \text{minimum} + (\text{maximum} - \text{minimum})/\left(1 + 10^{\wedge((\log XC_{50} - x)*\text{Hill slope})}\right)$$

where y is the membrane current, XC_{50} is the concentration of half-maximal efficacy, x is the logarithm of the agonist concentration. The fits were constrained to 1 and 0, except for the GABA and glycine concentration–response curves where the

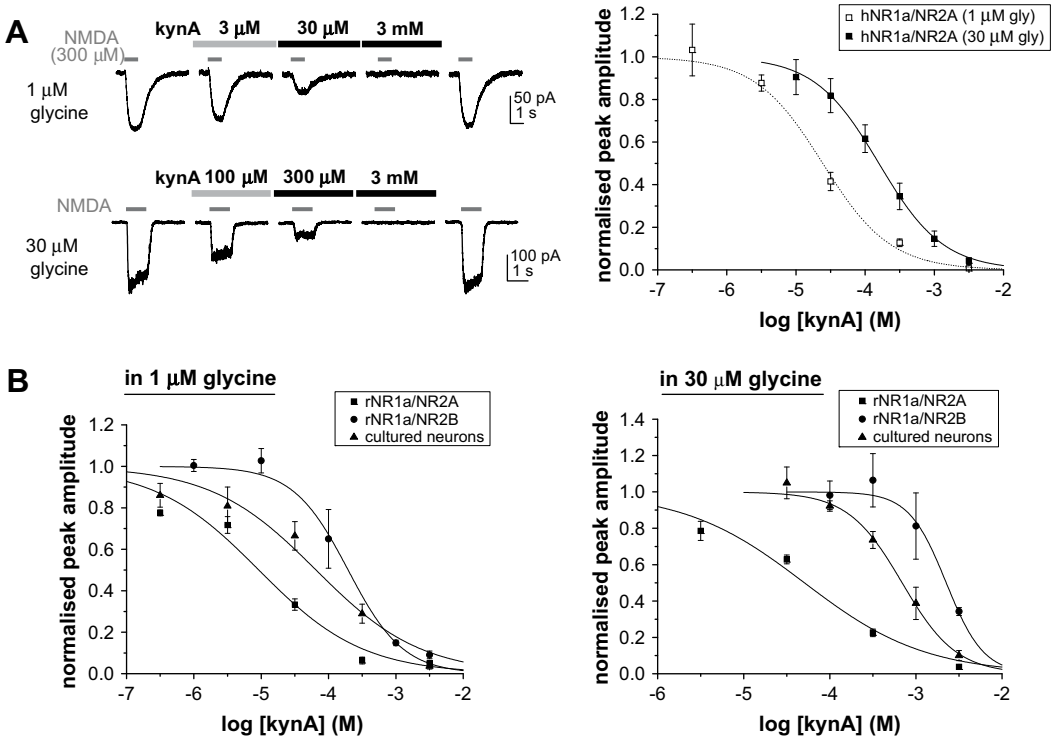

Fig. 1. Inhibition of NMDARs by kynA. A. *Left*, traces of typical recordings of HEK cells transiently transfected with human NR1a/NR2A in 1 μM (*top*) and 30 μM glycine (*bottom*). KynA reversibly inhibited currents evoked by NMDA (300 μM) in a concentration-dependent manner. *Right*, kynA inhibition curves for the human NR1a/NR2A receptor in the presence of 1 μM (*open*) and 30 μM glycine (*solid*; IC_{50} = 24.4 and 158.1 μM, respectively; n = 3–5 cells per data point). B. *Left*, KynA inhibition curves for the rat NR1a/NR2A (*squares*; IC_{50} = 9.2 μM), NR1a/NR2B (*circles*; IC_{50} = 195.4 μM) and native NMDA (*triangles*; IC_{50} = 65.2 μM) receptors in the presence of 1 μM glycine. *Right*, KynA inhibition curves for the rat NR1a/NR2A (*squares*; IC_{50} = 50.7 μM), NR1a/NR2B (*circles*; IC_{50} = 2243.9 μM) and native NMDA (*triangles*; IC_{50} = 690.2 μM) receptors in the presence of 30 μM glycine (n = 3–5 cells per data point).