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Short communication

Prolonged pre-exposure to 1-aminocyclopropanecarboxylic acid protects against subsequent glutamate toxicity in vitro

Yu Lin *, Joseph B. Long

Division of Neurosciences, Walter Reed Army Institute of Research, Building 40, Room 3077, Washington, DC 20307, USA

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Abstract

Sustained 20 h pre-exposure to 1 mM 1-aminocyclopropanecarboxylic acid (ACPC, which was removed 30 min before addition of 25 μM glutamate) significantly reduced the subsequent neurotoxicity of glutamate in cultured forebrain and cerebellar neurons. The magnitude of neuronal protection was further enhanced if the neurons pretreated with ACPC were re-exposed to ACPC during glutamate challenge. These results closely resemble earlier findings with cultured spinal cord neurons and indicate that these primary cell culture preparations might be suitable for the assessment of the mechanism(s) underlying chronic ACPC-induced modification of the NMDA receptor complex. © 1997 Published by Elsevier Science B.V.

Keywords: 1-Aminocyclopropanecarboxylic acid; Neuroprotection; Glutamate toxicity; Neuronal injury; Glycine receptor; NMDA receptor complex

1. Introduction

Due to the apparent co-requirement for glycine to activate the NMDA receptor-gated cation channels (Kleckner and Dingledine, 1988; Huettner, 1989; Benveniste and Mayer, 1991), antagonists acting at the strychnine-insensitive glycine receptor component of the NMDA receptor complex share many of the pharmacological actions of competitive NMDA receptor antagonists and use-dependent channel blockers (reviewed in Palfreyman and Baron, 1991; Carter, 1992). In addition to glycine receptor antagonists such as 7-chlorokynurenate (7-CK), glycinergic ligands with reduced efficacy (i.e., partial agonists) have also been demonstrated to block or reduce a variety of NMDA receptor-mediated actions in vivo and in vitro, presumably under conditions in which synaptic concentrations of glycine are at or near saturation. Among these compounds, the glycine partial agonist 1-aminocyclopropanecarboxylic acid (ACPC) is particularly noteworthy since it has been shown to protect against ischemic brain and spinal cord injury in vivo (Skolnick et al., 1989, 1992; Long and Skolnick, 1994; Zapata et al., 1996) through two fundamentally different mechanisms that are associated with two distinctly different dosing regimens. Specifically, in addition to being protective when administered at the time of the ischemic insult, ACPC was also shown to be protective when administered repeatedly during the week preceding injury. In the latter situation, the salutary effects of ACPC were evident despite undetectable brain and plasma concentrations of ACPC following a one day washout period immediately preceding the experiment (Von Lubitz et al., 1992), prompting the interpretation that chronically-administered ACPC reversibly desensitized the NMDA receptor complex by uncoupling glycine and glutamate binding sites.

Attempts to recreate the in vivo effects of chronically administered ACPC using neuronal cells in culture have yielded disparate results. For example, in contrast to the apparent desensitization to NMDA observed in vivo, ACPC sensitized cultured cerebellar granule cells to NMDA receptor agonists while reducing their responsiveness to ACPC and other glycine partial agonists. However, the sustained pre-exposure of primary cultures of rat spinal cord neurons to ACPC yielded changes more closely aligned with those noted in vivo, as the cells were rendered less responsive to the neurotoxic effects of NMDA (Lin and Long, 1996). The obviously divergent influences of chronic ACPC on cerebellar granule cells and on spinal cord neurons in these studies point to fundamentally different cellular characteristics that might mirror similar anatomical heterogeneity in the intact central nervous system. In these experiments, we assessed the impact of acute

* Corresponding author. Tel.: (1-202) 782-4259; Fax: (1-202) 782-6910.

and sustained exposures to ACPC on NMDA receptor-mediated injury using cells originating from the cortex and cerebellum of prenatal rat brain. We report that both acute coexposure and sustained pre-exposure of cells to ACPC protect against NMDA-induced cell injury in these neuronal culture preparations.

2. Materials and methods

The experimental methods used in this study have been reported previously (Lin and Long, 1996). Briefly, primary neuronal cell cultures were prepared from prenatal day 15 Sprague–Dawley rat embryos. After a 48 h incubation, cytosine arabinoside (10 μM) was added to inhibit non-neuronal cell division. Cells were used in experiments after 7 days in culture. ACPC, dissolved in neuronal culture medium, was added to the cultures to a final 1 mM concentration 20–24 h prior to the neurotoxicity assays. Half of the wells of each 48 well plate were pre-exposed to ACPC and cells in the remaining wells served as controls. Cell damage was quantitatively assessed 24 h after exposure to 25 μM glutamate using a tetrazolium salt colorimetric assay with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma, St. Louis, MO, USA). This dye is converted to an insoluble blue formazan product by living cells, which is then dissolved in acidified isopropanol and quantitated by absorbance intensity. Changes in cell viability are expressed relative to untreated control cells that were maintained on each plate. Each experimental condition was replicated in a minimum of 15 wells using cells obtained from at least 3 independent preparations. Differences in the cell viability among treatment groups were determined using one-way analysis of variance and the Newman–Keuls test (GB-STAT V5.3, Dynamic Microsystems, Silver Spring, MD, USA).

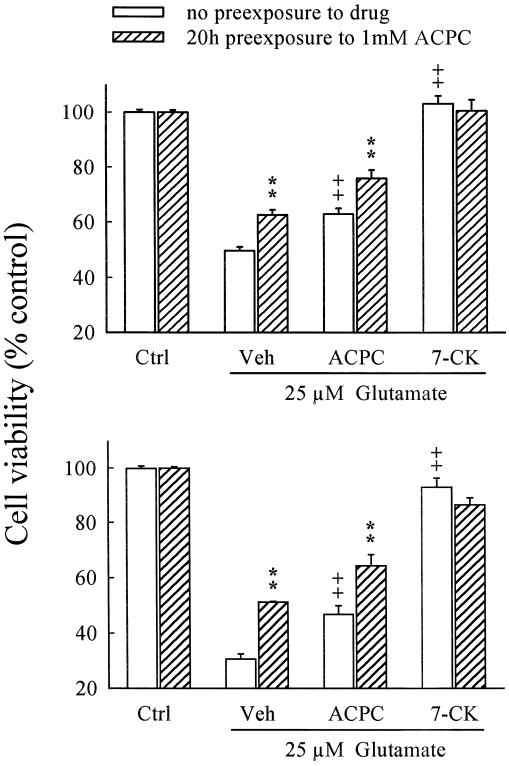

Fig. 1. Cultured forebrain (top panel) and cerebellar (bottom panel) cells were less severely damaged by 25 μM glutamate after 20 h preexposure to 1 mM ACPC (removed 30 min before glutamate addition) than the control cells not pre-exposed to ACPC. Co-exposure of cells to 1 mM ACPC during glutamate exposure also significantly reduced glutamate toxicity to a level comparable to that seen following sustained ACPC pretreatment. When the cells pre-exposed to ACPC were re-treated with ACPC during glutamate exposure/application, this protection was significantly greater than was produced by either treatment alone. The acute protective effects of 100 μM 7-CK were not appreciably diminished after ACPC pretreatment. $^{**}P < 0.01$, effects of sustained ACPC pretreatment in comparison to cells receiving no pretreatment. $++P < 0.01$, effects of acute applications of ACPC or 7-CK on glutamate toxicity.

3. Results

30 min exposures to 25 μM glutamate caused 50 and 70% loss of viability in forebrain and cerebellar cells, respectively. In both preparations, cell injury was nearly completely blocked by competitive (2-amino-5-phosphonopentanoic acid (APV, 200 μM)) and noncompetitive (dizocilpine maleate (MK-801, 1 μM)) inhibitors of the NMDA receptor complex (results not shown) and was also significantly reduced by cotreatment with 1 mM ACPC (Fig. 1). When added as a pretreatment for 20–24 h and then removed 30 min before exposure of the cells to 25 μM glutamate, 1 mM ACPC caused both forebrain and cerebellar cells to be significantly less responsive to the neurotoxic effects of 25 μM glutamate than control cells not pre-exposed to ACPC. As shown in Table 1, in fact, with 25 μM glutamate the protection resulting from pre-exposure to 1 mM ACPC was very similar to that produced by coexposure to 1 mM ACPC (23% and 29% protection in forebrain cultures; 26% and 26% in cerebellar cultures, respectively). In contrast to the findings of Boje et al. (1993), the acute neuroprotective effects of ACPC were not diminished after pre-exposure to ACPC; significant additional neuroprotection was evident in the

Table 1
Percentage protection induced by ACPC (1 mM) against glutamate (25 μM) toxicity[a]

Experimental conditions	Forebrain cultures (%)	Cerebellar cultures (%)
Preexposure to ACPC	23.0	29.0
Coexposure to ACPC	26.0	25.6
Combined exposures	48.5	52.4

[a] Percentage protection = (treatment − maximal injury) × 100%/control − maximal injury.

ACPC-pre-exposed neurons when they were re-exposed to 1 mM ACPC at the time of glutamate addition (48.5% protection in forebrain cultures and 52.4% in cerebellar cultures). The neuroprotective effects of 7-CK also were not significantly diminished after ACPC pre-exposure (Fig. 1).

4. Discussion

The results of this study are consistent with our earlier findings with spinal neuronal cultures in which ACPC antagonized excitotoxic phenomena mediated through the glutamate/NMDA receptor complex both acutely and following sustained pre-exposure (Lin and Long, 1996). In the previous study the possibility that residual ACPC remaining in the wells after media removal and replacement could account for protection following sustained ACPC exposure was ruled out because: (1) similar protection was not seen following shorter pre-exposure intervals and removal of ACPC and (2) lower concentrations of ACPC were ineffective as neuroprotectants with coexposure to 25 μM NMDA. This conclusion is further supported in the present study by the significant enhancement of the acute protective effects of an otherwise maximally effective 1 mM concentration of ACPC (unpublished results) after ACPC pre-exposure (Fig. 1a and b).

The results of these experiments clearly differ from those of Boje et al. (1993) and Fossom et al. (1995a,b) in which sustained exposure of cultured cerebellar granule cells to ACPC was not neuroprotective and instead significantly diminished the acute protective effects of ACPC and other glycine partial agonists, eg. D-cycloserine, 3-amino-1-hydroxy-2-pyrrolidone (HA-966) and significantly enhanced the potencies of NMDA receptor agonists (eg. glutamate and NMDA). These differences presumably can be attributed to the fundamentally different characteristics of the cells contained in the different preparations, which reflect in large part their different origins. Specifically, in contrast to our use of forebrain and cerebellar cells prepared from prenatal day 15 rat embryos, the earlier studies were conducted with cerebellar granule cells prepared from postnatal 6–8 day old rat pups. Due to the well-defined temporal differences in the morphogenesis and proliferation of different types of cerebellar neurons, cultures prepared at these different developmental stages will have very different neuronal constituents and ensuing organizational interactions in culture (Altman and Bayer, 1978). Although they provide a well-characterized NMDA receptor complex that is modulated by glycinergic ligands, the pharmacological responsiveness of the enriched cerebellar granule cell cultures used by Boje et al. (1993) and Fossom et al. (1995a) differed appreciably in several regards from that seen in vivo and in other primary cultures. For example, although glutamate caused dose-dependent neuronal cell damage that was blocked by NMDA receptor antagonists, NMDA itself failed to injure granule cell preparations in concentrations up to 1 mM (Boje et al., 1993). NMDA did however cause other predictable cellular responses that were mediated through the NMDA receptor complex, such as elevations in intracellular calcium (Fossom et al., 1995a). In contrast, with prenatally-obtained forebrain and cerebellar neuronal cultures we have observed both glutamate and NMDA to be excitotoxic and to elevate intracellular calcium (unpublished results). Possibly, as discussed by Fossom et al. (1995a,b), recreation of the pharmacological effects of ACPC that have been described in vivo may require a higher level of organizational complexity and neuronal diversity than is provided by relatively homogeneous, highly enriched granule cell cultures. The present findings confirm that along with spinal cord neurons, primary cultures of prenatal forebrain and cerebellar neurons more closely model the in vivo situation and potentially provide a useful means to ascertain the cellular mechanism underlying ACPC-induced neuroprotective desensitization.

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In conducting the research described in this report, the investigator(s) adhered to the 'Guide for the Care and Use of Laboratory Animals,' as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The views of the author(s) do not purport to reflect the position of the Department of the Army or the Department of Defense (para 4-3, AR 360-5).

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