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European Journal of Pharmacology 318 (1996) 491–496

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Acute or prolonged exposure to 1-aminocyclopropanecarboxylic acid protects spinal neurons against NMDA toxicity [1]

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Received 4 July 1996; revised 1 October 1996; accepted 8 October 1996

Abstract

1-Aminocyclopropanecarboxylic acid (ACPC) is a high affinity partial agonist for the glycine binding site within the NMDA receptor complex. Chronic treatment with ACPC in vivo appears to reversibly desensitize the NMDA receptor complex, prompting suggestions that it might provide an effective means of ameliorating degenerative mechanisms mediated through this ligand-gated ion channel. In the present experiments, cultured rat spinal cord neurons were used to further examine the effects of acute and sustained ACPC exposures on *N*-methyl-D-aspartate (NMDA)-induced neurotoxicity. Cell damage was quantitatively assessed using a tetrazolium salt colorimetric assay. With coincubation, 1 mM ACPC significantly reduced the neuronal cell damage caused by 30 min exposure to 25 or 50 μM concentrations of NMDA, but, in contrast to other competitive and non-competitive NMDA receptor antagonists (D-(−)-2-amino-5-phosphonovaleric acid (APV), dizocilpine maleate (MK-801) and 7-chlorokynurenic acid (7-CK)), it failed to alter the cell injury induced by 100 μM NMDA. The protective effect of ACPC was competitively abolished by coaddition of glycine, verifying that it was mediated through glycine binding sites. Sustained 20 h exposure to 1 mM ACPC (which was removed 30 min before addition of 25 μM NMDA) also caused cells to be significantly less responsive to the neurotoxic effects of NMDA. Pre-exposure to ACPC for shorter intervals (< 1 h) failed to alter subsequent NMDA toxicity. Acute or sustained exposures to ACPC alone did not affect cell viability. These results support earlier indications that: (1) ACPC provides an effective means of antagonizing excitotoxic phenomena, and (2) sustained exposure to ACPC desensitizes the NMDA receptor complex.

Keywords: ACPC (1-aminocyclopropanecarboxylic acid); Neuroprotection; NMDA receptor; Toxicity; Neuronal injury; Glycine; Excitatory amino acid

1. Introduction

Activation of the NMDA receptor complex has been proposed to substantially contribute to the neuronal degeneration associated with ischemic and traumatic central nervous system injury and a variety of neurodegenerative disorders (for review, see Lipton and Rosenberg, 1993). Hence, interruption of the events mediated through this ligand-gated ion channel has been widely pursued as a therapeutic strategy. Due to the apparent corequirement for glycine to activate NMDA receptor-gated cation channels (Benveniste and Mayer, 1991; Huettner, 1989; Kleckner and Dingledine, 1988), antagonists such as 7-chlorokynurenic acid (7-CK), which act at strychnine-insensitive glycine receptors, also can function as antagonists of the NMDA receptor complex and share many of the pharmacological actions of both competitive NMDA receptor antagonists and use-dependent channel blockers (reviewed in Carter, 1992; Palfreyman and Baron, 1991).

Glycinergic ligands with reduced efficacy (i.e., partial agonists) can also act as functional NMDA receptor antagonists under conditions in which synaptic concentrations of glycine are presumed to be at or near saturation. For example, the glycine partial agonist 1-aminocyclopropanecarboxylic acid (ACPC) has been demonstrated to block or reduce a variety of NMDA receptor-mediated actions both in vivo and in vitro (Boje et al., 1993; Fossom et al., 1995a,b; Skolnick et al., 1989, 1992; Zapata et al., 1996). Moreover, this compound has been shown to pro-

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[1] 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).

Published by Elsevier Science B.V.
PII S0014-2999(96)00811-4

tect against ischemic brain and spinal cord injury in vivo and to block NMDA receptor-mediated neurotoxicity in vitro (Boje et al., 1993; Fossom et al., 1995a,b; Long and Skolnick, 1994; Von Lubitz et al., 1992). In the central nervous system ischemic injury models in vivo, ACPC's protective effects appeared to result from fundamentally different mechanisms that were associated with two distinctly different dosing regimens. Specifically, ACPC was shown to be protective either when administered at the time of ischemic insult or when administered repeatedly during the week preceding injury. With the latter dosing regimen, the salutary effects of the chronically administered ACPC were evident despite undetectable brain and plasma concentrations of ACPC following a one day washout period immediately preceding the experiment, prompting the interpretation that chronic administration of ACPC reversibly desensitizes the NMDA receptor complex by uncoupling glycine and glutamate binding sites (Von Lubitz et al., 1992).

In contrast to the findings from studies in vivo, the sustained 24 h exposure of primary cultures of rat cerebellar granule cells to ACPC and other glycinergic ligands with partial or full agonist characteristics resulted in an attenuation of the neuroprotective effects of glycine partial agonists such as ACPC and (±)-3-amino-1-hydroxy-2-pyrrolidone (HA-966) against NMDA receptor-mediated neuronal cell injury (Boje et al., 1993). Subsequent evaluations with this model further revealed that sustained exposure to ACPC also caused significant increases in the potency of NMDA to elevate intracellular Ca^{2+} and cGMP, and similarly enhanced the potency of glutamate to cause neuronal cell damage (Fossom et al., 1995b). Thus, cultured rat cerebellar granule cells clearly respond to prolonged exposure to ACPC, but the divergence in these desensitization responses from those observed in vivo reveal that these cells do not provide a means to model in vitro the mechanisms underlying the neuroprotective effects of chronically administered ACPC.

Based upon the previous demonstration of the protective effects of ACPC with dynorphin A-induced ischemic spinal cord injury in vivo (Long and Skolnick, 1994), in the present study primary cultures of rat spinal cord neurons were used to assess the impact of acute and sustained exposures to ACPC on NMDA receptor-mediated cell injury. We report that both acute coexposure and sustained preexposure of cells to ACPC protect against NMDA-induced cell injury.

2. Materials and methods

2.1. Cell cultures

Primary neuronal cell cultures were prepared from spinal cords removed from prenatal day 15 Sprague-Dawley rat embryos. Following trituration, cells were plated at a density of 5×10^5 cells/well in 48 well culture plates pre-coated with poly-L-lysine. Cultures were maintained in a medium containing equal parts of Eagle's basal media (without glutamine) and Ham's F12 K media supplemented with 10% heat-inactivated horse serum, 10% fetal bovine serum, glucose (600 μg/ml), glutamine (100 μg/ml, penicillin (50 units/ml), and streptomycin (50 μg/ml). After 48 h, cytosine arabinoside (10 μM) was added to inhibit non-neuronal cell division. Cells were used in experiments after 7 days in culture.

2.2. Viability assessments

For viability experiments, media was replaced with Locke's solution from which magnesium chloride and glucose were omitted. After one rinse with this solution, cells were exposed to drugs and/or NMDA for 30 min at 37°C. Locke's solution was then replaced with minimal essential medium and incubation of the cultures was continued for another 18–20 h in a 5% CO_2 incubator at 37°C. For experiments evaluating the effect of sustained exposure to ACPC, 1 mM ACPC, dissolved in neuronal cultural media, was added directly to the cultures for 20–24 h prior to the neurotoxicity assays. In these experiments, half of the wells of each plate were pre-exposed to ACPC, and cells in the remaining wells served as controls.

Cell damage was quantitatively assessed using a tetrazolium salt colorimetric assay with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma, St. Louis, MO, USA). Briefly, this dye was added to each well (final concentration of 0.15 mg/ml) and was converted to an insoluble blue formazan product by living cells. Cells were incubated with MTT for 1 h at 37°C. When the assay was terminated, the dye was solubilized by acidified isopropanol (0.1 M HCl in isopropanol) and the absorbance intensity (540 nM) of each solution measured in a 96-well plate reader. To normalize for differences in the absorbance values of MTT color reaction product among different culture plates and assays following NMDA exposure, values were expressed relative to untreated control cells and NMDA-treated cells that were maintained on each plate, and a percentage change in cell viability was calculated. Each experimental condition was replicated in a minimum of 15 wells using cells obtained from at least 3 independent preparations.

2.3. Chemicals

1-Aminocyclopropanecarboxylic acid (ACPC) was obtained from Research Organics (Cleveland, OH, USA), 7-chlorokynurenic acid (7-CK), and MK-801 from Research Biochemicals International (Natick, MA, USA), *N*-methyl-D-aspartic acid (NMDA) and MTT from Sigma, D(−)-2-amino-5-phosphonovaleric acid (APV) from Cambridge Research Biochemicals (Cambridge, UK).

2.4. Data analysis

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). ED_{50} values and their 95% confidence intervals were determined using the computer program described by Tallarida and Murray (1986).

3. Results

Thirty minutes exposures to NMDA caused concentration-dependent toxicity to spinal neurons with an ED_{50} (and 95% confidence intervals) of 25 (14–45) μM (Fig. 1). A maximum 47% loss of viability resulted from exposure of cells to NMDA concentrations ≥ 100 μM relative to the MTT absorbance values recorded in control cells exposed to vehicle. These injuries were significantly eliminated by both competitive and non-competitive antagonists of the NMDA receptor complex (Fig. 2); cotreatment with the competitive NMDA receptor antagonist APV (500 μM) completely blocked neuronal injury resulting from 100 μM NMDA.

ACPC alone did not affect cell viability in concentrations up to 1 mM. When added immediately before NMDA, 1 mM ACPC significantly protected against cell injury induced by 25 and 50 μM NMDA (Fig. 3A), but in contrast to the glycine receptor antagonist 7-CK, failed to block the neurotoxicity of 100 μM NMDA (Fig. 2). Lower concentrations of ACPC were ineffective at improving cell viability following 30 min exposures to 25, 50, or 100 μM NMDA (Fig. 3B). The protective effects of ACPC were abolished by coaddition of glycine in a dose-related manner. Specifically, the protective effects of 1 mM ACPC against 25 and 50 μM NMDA were eliminated by 1000 and 10 μM glycine, respectively (Fig. 4). It is noteworthy

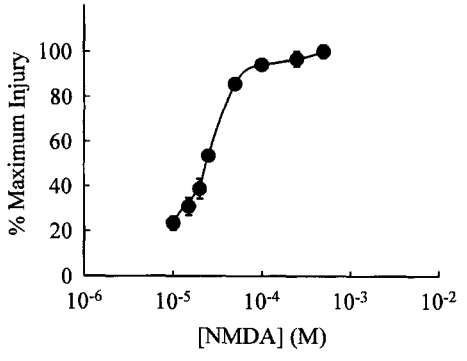

Fig. 1. Concentration-dependent injuries were evident in spinal neurons following 30 min exposures to NMDA with an ED_{50} (and 95% confidence intervals) of 25 (14–45) μM. NMDA toxicity is expressed as percentage of the maximal cell death induced by 500 μM NMDA. Comparable toxicities were seen with NMDA concentrations ≥ 100 μM. $n \geq 18$ wells per treatment condition.

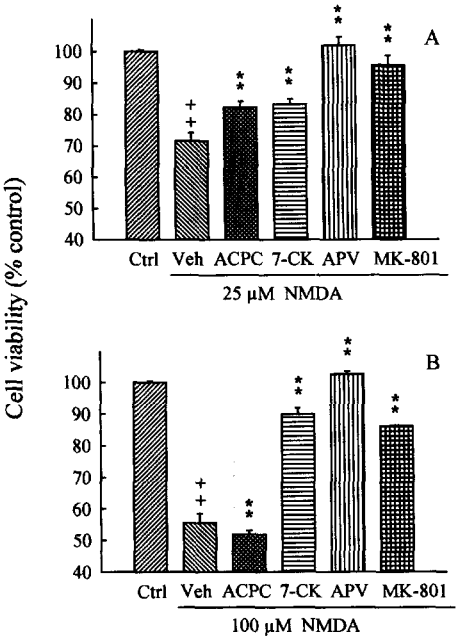

Fig. 2. The effects of competitive and noncompetitive NMDA receptor antagonists on cell injuries resulting from exposure to 25 (A) and 100 (B) μM NMDA. In this and the following figures, cell viability is expressed relative to the MTT measurements made in control (vehicle-treated) cells maintained on each plate. Although protective against 25 μM NMDA, in contrast to the other agents ACPC failed to reduce the neuronal injury induced by 100 μM NMDA. (ACPC, $n = 99$; 7-CK, $n = 51$; APV, $n = 27$; MK-801, $n = 27$). $^{++}$ $P < 0.01$, NMDA toxicity in comparison to vehicle treatment. ** $P < 0.01$, effect of antagonists on NMDA toxicity.

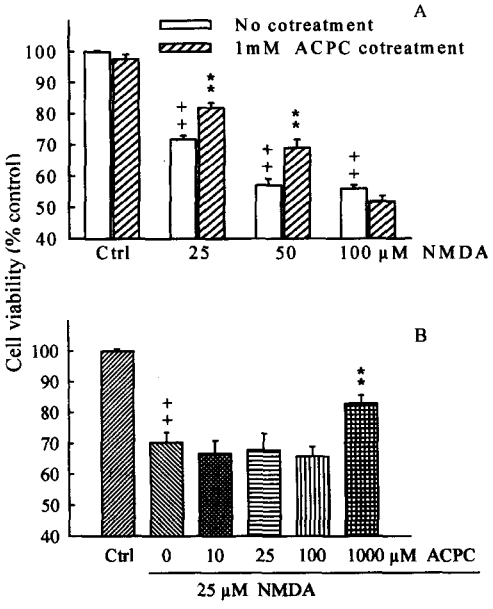

Fig. 3. (A) ACPC alone was not toxic to spinal neurons ($n = 33$). Cotreatment with 1 mM ACPC reduced injury resulting from exposure to 25 and 50 μM NMDA ($n = 99$ and 54, respectively), but failed to protect neurons exposed to 100 μM ($n = 39$). (B) Concentrations of ACPC less than 1 mM failed to protect cells coexposed to 25 μM NMDA ($n = 27$). $^{++}$ $P < 0.01$, NMDA toxicity in comparison to vehicle treatment. ** $P < 0.01$, effect of ACPC on NMDA toxicity.

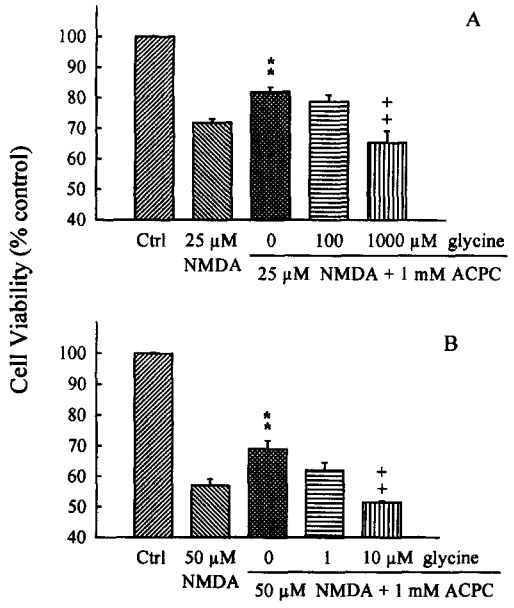

Fig. 4. Glycine reversal of ACPC protective effects against 25 (A) and 50 (B) μM NMDA. Less glycine was required to restore toxicity with 50 than with 25 μM NMDA. (A) 0.1 mM glycine treatment, $n = 42$; 1 mM glycine treatment, $n = 18$. (B) 1 μM glycine treatment, $n = 36$; 10 μM glycine treatment, $n = 30$. $^{**}P < 0.01$, effect of ACPC on NMDA toxicity. $^{++}P < 0.01$, effect of glycine to restore NMDA toxicity. Comparisons were made in cells where glycine was either omitted (cross-hatch bar) or inluded with the ACPC and NMDA.

that 10 μM glycine failed to potentiate the toxic effects of 50 μM NMDA (results not shown). Thus, it is possible to abolish the protective effects of ACPC under conditions where glycine does not potentiate NMDA toxicity.

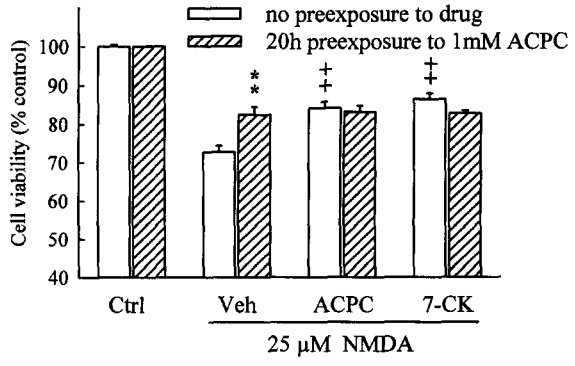

Fig. 5. Desensitization to NMDA toxicity after sustained preexposure to ACPC. ACPC was removed 30 min before exposure to 25 μM NMDA. Cells preexposed to 1 mM ACPC (filled column, $n = 33$) were less severely damaged by NMDA in comparison to the control cells not preexposed to ACPC (open column, $n = 33$). Although 1 mM ACPC ($n = 33$) and 100 μM 7-CK ($n = 18$) protected against NMDA-induced neuronal loss in the naive cultures not preexposed to ACPC, no additional protection was evident when these drugs were present during NMDA exposure in the ACPC-pretreated cultures. Experiments were carried out in plates where half of the wells were pretreated for 20 h with 1 mM ACPC and the other half were exposed to the vehicle neuronal culture medium alone. $^{**}P < 0.01$, effect on NMDA toxicity in ACPC pretreated cells in comparison to no drug treated cells. $^{++}P < 0.01$, effect of acute treatment with antagonists on NMDA toxicity in comparison to NMDA treatment alone.

When added as a pretreatment 20–24 h before exposure of cells to 25 μM NMDA, 1 mM ACPC caused cells to be significantly less responsive to the neurotoxic effects of NMDA than were control cells not preexposed to ACPC. In these experiments, ACPC was dissolved in neuronal culture medium, and a 30 μl aliquot was added to yield a 1 mM ACPC concentration in each well. The remaining wells on each plate were exposed to neuronal culture medium alone. Thirty minutes before addition of NMDA, cell culture medium was removed from each well and replaced with Locke's solution, and cells were treated in a manner identical to that used in the experiment described above. Pre-exposure of cells to ACPC for shorter intervals (< 1 h) failed to alter subsequent toxicity of 25 μM NMDA (results not shown). In addition, following the 20–24 h ACPC pretreatment, acute coexposure to either 1 mM ACPC or 100 μM 7-CK did not provide any additional protection against cell injuries induced by 25 μM NMDA beyond that resulting from the ACPC preexposure alone (Fig. 5).

4. Discussion

The results of this study support previous indications that ACPC can antagonize excitotoxic phenomena mediated through the NMDA receptor complex. However, in contrast to APV, MK-801 and 7-CK, the protective effects of ACPC were restricted to 25 and 50 μM concentrations of NMDA and caused only a partial reversal of the cell damage associated with these submaximally neurotoxic concentrations of NMDA. Based upon the previous descriptions of this compound as a partial agonist acting at strychnine-insensitive glycine sites of the NMDA receptor complex (Marvizon et al., 1989; Priestley and Kemp, 1994; Watson and Lanthorn, 1990), its significant, albeit limited antagonism of NMDA neurotoxicity is in fact entirely predictable and parallels its restricted efficacy as an antagonist with several other NMDA-mediated endpoints, including glutamate neurotoxicity in cultured cerebellar granule cells (Fossom et al., 1995b).

Addition of exogenous glycine reversed the neuroprotective effects of ACPC, implicating the involvement of strychnine-insensitive binding sites in these pharmacological actions. In related experiments, the addition of exogenous glycine also significantly potentiated the neurotoxic effects of 25 μM NMDA (manuscript in preparation), revealing that under our standard assay conditions, glycine is normally present in subsaturating concentrations. However, as reported by others (Boje et al., 1993), we observed that 1 or 10 μM concentrations of glycine failed to exacerbate the neuronal injuries caused by more toxic concentrations (50 or 100 μM) of NMDA, possibly revealing that the relative contribution of the glycine binding site to the neurotoxicity mediated through the NMDA receptor complex is conditionally dependent upon the coexisting

level of NMDA receptor stimulation, and although still essential, is reduced under excitotoxic conditions where the NMDA receptor per se is maximally activated. The inability of ACPC to antagonize a maximally toxic (100 μM) concentration of NMDA supports this interpretation, and is also consistent with a series of other observations summarized by Fossom et al. (1995b), in which the apparent efficacy of ACPC generally appeared to depend upon excitatory amino acid concentration, and was lost at high glutamate or NMDA concentrations. This condition may also account for the apparent requirement for greater concentrations of glycine to reverse the neuroprotective effects of ACPC in the presence of 25 μM NMDA relative to that required with 50 μM NMDA (Fig. 4).

Despite its limited neuroprotective efficacy, as a partial agonist ACPC might have several therapeutic advantages over full antagonists at the strychnine-insensitive glycine site (e.g., 7-CK) as well as ligands targetting other components of the NMDA receptor complex. Specifically, ACPC should most effectively antagonize NMDA receptor function when and where synaptic concentrations of glycine are at or near saturation. Under pathophysiological conditions such as stroke or brain ischemia, where glycine and glutamate concentrations appear to be selectively elevated in disrupted brain regions (Globus et al., 1991; Graham et al., 1990; Hillered et al., 1989 and Takagi et al., 1993), the maximal effects of ACPC should be similarly localized. Consequently, deleteriously elevated NMDA receptor activity may be attenuated with a minimal disruption of NMDA receptor function elsewhere in the brain. In addition, as a partial agonist ACPC does not completely block NMDA receptor activity as do the full antagonists targetting this receptor complex. As a result, untoward NMDA receptor activity may be therapeutically modulated rather than eliminated, and fewer drug side effects may be expected. The ability of ACPC to produce glycine-reversible neuroprotection in vivo (Long and Skolnick, 1994; Von Lubitz et al., 1992) suggests that, under pathophysiological conditions, synaptic glutamate and glycine concentrations are evidently in the range permitting ACPC to act as a low-to-moderate efficacy antagonist of excitotoxicity mediated through the NMDA receptor complex.

In addition to the acute neuroprotection resulting from coexposure to ACPC during NMDA treatment, pre-exposure of spinal cord neurons to 1 mM ACPC for 20–24 h preceding the addition of 25 μM NMDA significantly reduced NMDA toxicity. Since the ACPC was removed 30 min before addition of NMDA, the diminished toxicity presumably resulted from a drug-induced alteration in the responsiveness of the cells (i.e., desensitization). It is unlikely that residual ACPC potentially remaining in the wells after media removal and replacement could account for this protection because: (1) similar protection was not seen following shorter preexposure intervals and removal of ACPC, and (2) lower concentrations of ACPC were ineffective as neuroprotectants with coexposure to 25 μM NMDA (Fig. 3B). Thus, these results point to a time-dependent change in responsivity to NMDA that is associated with prolonged exposure to ACPC.

In earlier work with glutamate-induced injuries in cultured cerebellar granule cells, Boje et al. (1993) observed that after 20–24 h sustained exposure to glycinergic ligands, the protective effects of ACPC and HA-966 were attenuated, whereas the neuroprotective effects of relatively high concentrations of APV and MK-801 were unaltered. In subsequent studies, Fossom et al. (1995b) further reported that sustained exposure of cerebellar granule cells to 1 mM ACPC also significantly increased the neurotoxic potency of glutamate, and caused parallel increases in the potency of NMDA to increase intracellular Ca^{2+} and to increase cGMP. The obviously divergent influences of chronic ACPC on spinal cord neurons in the present experiments and on cerebellar granule cells in these earlier studies point to fundamentally different properties of these cells that might mirror similar anatomical heterogeneity in the intact central nervous system.

As the result of both neurochemical and molecular biological assessments, insights have been gained into the heterogeneous nature of NMDA receptors and have revealed functional differences that correspond with differences in the heteromeric combinations of NMDA receptor complex subunits. For example, in *Xenopus* ooytes expressing NMDA-R1 and -2C subunits the respective potencies for glycine and glutamate were approximately 25- and 6.5-fold higher than were seen with receptors comprised of the NMDA-R1 and and -2A subunits (Wafford et al., 1993). Thus, it is conceivable that the differing pharmacological outcomes in these experiments may reflect prominent differences in the underlying molecular compositions of the NMDA receptor complexes in the different cell preparations. Furthermore, the pharmacological alterations induced in cerebellar granule cells by sustained exposure to ACPC were associated with an approximately 2.5-fold increase in expression of RNA encoding the NMDA receptor 2C subunit without concommitant changes in levels of mRNA encoding the NMDA receptor 2A, 2B, or 1 subunits (Fossom et al., 1995a). Thus, drug-induced changes in NMDA receptor subunit composition may also provide a molecular basis for the pharmacologically induced alteration in receptor responsiveness seen in those earlier experiments. The mechanism(s) underlying the changes described in the present report have not yet been addressed, but conceivably could also involve receptor subunit changes and/or alterations in affinities at glycine or NMDA binding sites (Nowak et al., 1993).

In summary, the results of the present study demonstrate that, in addition to protection arising from coexposure to ACPC, sustained preexposure to ACPC also protected spinal cord neurons against NMDA receptor-mediated injury. These effects of ACPC in vitro parallel those seen following chronic administration of ACPC in vivo, and potentially indicate that cultured spinal cord neurons

might provide a useful means to model this apparent neuroprotective desensitization. Based upon the neurobiological importance of NMDA receptor-gated ion channels and the associated current clinical interests in ACPC, additional studies addressing the underlying basis for ACPC-induced chronic modification of NMDA effector mechanisms might provide therapeutic insights relevant to disorders linked to excessive activation of this receptor complex.

References

Benveniste, M. and M. Mayer, 1991, Kinetic analysis of antagonist action at *N*-methyl-D-aspartic acid receptors – two binding sites each of glutamate and glycine, Biophys. J. 59, 560.

Boje, K.M., G. Wong and P. Skolnick, 1993, Desensitization of the NMDA receptor complex by glycinergic ligands in cerebellar granule cell cultures, Brain Res. 603, 207.

Carter, A.J. 1992, Glycine antagonists: regulation of the NMDA receptor-channel complex by the strychnine-insensitive glycine site, Drugs Future 17, 595.

Fossom, L.H., A.S. Basile and P. Skolnick, 1995a, Sustained exposure to 1-aminocyclopropanecarboxylic acid, a glyine partial agonist, alters *N*-methyl-D-aspartate receptor function and subunit composition, Mol. Pharmacol. 48, 981.

Fossom, L.H., D.K.J.E. Von Lubitz, R.C.-S. Lin and P. Skolnick, 1995b, Neuroprotective actions of 1-Aminocyclopropanecarboxylic acid (ACPC): a partial agonist at strychnine-insensitive glycine sites, Neurol. Res. 17, 265.

Globus, M.Y.-T., R. Busto, E. Martinez, I. Valdes, W.D. Dietrich and M.D. Ginsberg, 1991, Comparative effect of transient global ischemia on extracellular levels of glutamate, glycine, and γ-aminobutyric acid in vulnerable and nonvulnerable brain regions in the rat, J. Neurochem. 57, 470.

Graham, S.H., K. Shiraishi, S.S. Simon and A.I. Faden, 1990, Changes in extracellular amino acid neurotransmitters produced by focal cerebral ischemia, Neurosci. Lett. 110, 124.

Hillered, L., A. Hallstrom, S. Segersvard, L. Persson and U. Ungerstedt, 1989, Dynamics of extracellular metabolites in the striatum after middle cerebral artery occlusion in the rat monitored by intracerebral micordialysis, J. Cereb. Blood Flow Metab. 9, 607.

Huettner, J.E., 1989, Indole-2-carboxylic acid: a competitive antagonist of potentiation by glycine at the NMDA receptor, Science 243, 1611.

Kleckner, N.W. and R. Dingledine, 1988, Requirement for glycine in activation of NMDA-receptors expressed in Xenopus oocytes, Science 241, 835.

Lipton, S.A. and P.A. Rosenberg, 1993, Excitatory amino acids as a final common pathway for neurologic disorders, New Engl. J. Med. 330, 613.

Long, J.B. and P. Skolnick, 1994, 1-Aminocyclopropanecarboxylic acid protects against dynorphin A-induced spinal injury, Eur. J. Pharmacol. 261, 295.

Marvizon, J.C.G., A. Lewin and P. Skolnick, 1989, 1-Aminocyclopropanecarboxylic acid: a potent and selective ligand for the glycine modulatory site of the *N*-methyl-D-aspartate receptor complex, J. Neurochem. 52, 992.

Nowak, G., R. Trullas, R.T. Layerr, P. Skolnick and I.A. Paul, 1993, Adaptive changes in the *N*-methyl-D-aspartate receptor complex after chronic treatment with imipramine and 1-aminocyclopropanecarboxylic acid, J. Pharmacol. Exp. Ther. 265, 1380.

Palfreyman, M.G. and B.M. Baron, 1991, Non-competitive NMDA antagonists, acting on the glycine site, in: Pharmacology and Therapeutics: Excitatory Amino Acid Antagonists, ed. B.S. Meldrum (Blackwell, London) p. 101.

Priestley, T. and J.A. Kemp, 1994, Kinetic study of the interections between the glutamate and glycine recognition sites on the *N*-methyl-D-aspartic acid receptor complex, Mol. Pharmacol. 46, 1191.

Skolnick, P., J.C.G. Marvizon, B.W. Jackson, J.A. Monn, K.C. Rice and A.H. Lewin, 1989, Blockade of *N*-methyl-D-aspartate induced convulsions by 1-aminocyclopropanecarboxylates, Life Sci. 45, 1647.

Skolnick, P., R. Miller, A. Young, K. Boje and R. Trullas, 1992, Chronic treatment with 1-Aminocyclopropanecarboxylic acid desensitizes behavioral responses to compounds acting at the *N*-methyl-D-aspartate receptor complex, Psychopharmacology 107, 489.

Takagi, K.M.D. Ginsberg, M.Y.-T. Globus, W.D. Dietrich, E. Martinez, S. Kraydieh and R. Busto, 1993, Changes in amino acid neurotransmitters and cerebral blood flow in the ischemic penumbra region following middle cerebral artery occlusion in the rat: correlation with histopathology, J. Cereb. Blood Flow Metab. 13, 575.

Tallarida, R.J. and R.B. Murray, 1986, Manual of Pharmacological Calculations (Springer-Verlag, New York, NY).

Von Lubitz, D.K.J.E., R.C.-S. Lin, R.J. McKenhzie, T.M. Devlin, R.T. McCabe and P. Skolnick, 1992, A novel treatment of global cerebral ischaemia with a glycine partial agonist, Eur. J. Pharmacol. 219, 153.

Wafford, K.A., C.J. Bain, B. Le Bourdelles, P.J. Whiting and J.A. Kemp, 1993, Preferential co-assembly of recombinant NMDA receptors composed of three different subunits, NeuroReport 4, 1347.

Watson, G.B. and T.H. Lanthorn, 1990, Phamacological characteristics of cyclic homologues of glycine at the *N*-methyl-D-aspartate receptor-associated glycine site, Neuropharmacology 29, 727.

Zapata, A., J.L. Capdevila, E. Viu and R. Trullas, 1996, 1-Aminocyclopropanecarboxylic acid reduces NMDA-induced hippocampal neurodegeneration in vivo, NeuroReport 7, 397.