Premium Reasons

Journal of Theoretical & Experimental Pharmacology

ISSN (electronic): 1916-6958

2010; Volume 1; Number 2 (May-August): 72-75

The *Journal of Theoretical & Experimental Pharmacology* is an open-access journal published electronically by PremiumReasons®, located in Toronto, Ontario, Canada.

Published manuscripts are peer-reviewed by scientists with proven reputation in their field. Substantial efforts are made to publish only those manuscripts that properly justified the aim of the study, used appropriate methods, adequately summarized the results, and provided a sufficient analysis of the literature in comparison to the findings of the study. However, manuscripts published by the journal represent the sole opinion of the authors. PremiumReasons®, the Editor or the Editorial Board of the *Journal of Theoretical & Experimental Pharmacology* cannot assume any responsibility for the procedures, methods, chemical compounds, drugs, doses, statements of facts, or opinions expressed in the manuscripts, or any involuntary or intentional failure to disclose conflicts of interests. In addition, selected manuscripts may discuss investigational drugs or unlabeled uses of approved medications, or use of devices that had not been yet approved by regulatory agencies.

Finally, in order to promote and encourage environmental awareness, PremiumReasons® invites the readers of the *Journal of Theoretical & Experimental Pharmacology* to use the electronic version of the manuscripts rather than printing hard copies of the documents.

-.-.-.-.-.-.-.-.-

Premium Reasons

Journal of Theoretical & Experimental Pharmacology

ISSN 1916-6958
2010; Volume 1 (Number 2): Pages 72-75

ORIGINAL RESEARCH

Neuroprotective effects of glycine in rats with permanent cerebral ischemia

Rebeca URIBE-ESCAMILLA[a], Krystell PADILLA-MARTÍN[a], Angélica GONZÁLEZ-MACIEL[b], Emilio ARCH-TIRADO[a], Alejandro A. NAVA-OCAMPO[c,d], Alfonso ALFARO-RODRÍGUEZ[a,*].

[a]Laboratory of Neurochemistry, National Institute of Rehabilitation, Secretaría de Salud, México DF, Mexico, [b]Laboratory of Electronic Microscopy, National Institute of Pediatrics, Secretaría de Salud, México DF, Mexico, [c]Department of Pharmacology & Toxicology, Faculty of Medicine, University of Toronto, Toronto ON, Canada, [d]PharmaReasons, Toronto ON, Canada

*Corresponding author: alfa1360@yahoo.com.mx

ABSTRACT

Objective: The aim of this study was to evaluate the neuroprotective effects of glycine in an experimental animal model of permanent brain ischemic injury. Glycine is an inhibitory neurotransmitter amino acid that acts as neuromodulator of N-methyl-D-aspartate (NMDA) receptors, critically involved in the process of ischemic brain injury. **Methods:** Twenty-four Wistar rats, 20 days old, were randomly allocated to one of the following three groups. In Group 1: rats underwent a sham surgery where the left carotid was visualized only; in Group 2, rats underwent permanent left carotid occlusion and received treatment with placebo; and in Group 3, rats received glycine i.p. (40 mMol kg^{-1} 24 h^{-1}) for 30 days post-unilateral carotid occlusion. Thirty days post-surgery, the animals were sacrificed and brains were examined by histopathology. **Results:** In Group 1, normal brain histopathology was observed. In Group 2, tortuous capillary vessels were observed in the brain in addition to necrotic areas in hippocampus, medial vestibular nucleus, piriform and cerebral cortex, and thalamus. In Group 3, histopathological alterations were restricted to thalamus. **Conclusions:** Glycine administered i.p. to rats with permanent left carotid occlusion limited the ischemic brain damage probably by increasing the neurological availability of glycine concentration enough to prevent the desensitization of NMDA receptors and consequently altering the cascade of events that lead to cellular death.

Key words

Brain ischemia; Glycine; Neuroprotective agents.

RÉSUMÉ

Objectif: Évaluation des effets neuroprotecteurs de la glycine dans un modèle animal expérimental d'ischémie cérébrale permanente. La glycine est un acide aminé neurotransmetteur inhibiteur qui agit comme neuromodulateur des récepteurs au N-méthyl-D-aspartate (NMDA) impliqués dans le processus d'ischémie cérébrale. **Méthodes:** Vingt-quatre rats Wistar âgés de 20 jours ont été assignés *at random* à un des trois groupes suivants. Groupe 1: les rats ont subi une chirurgie factice où la carotide gauche était uniquement visualisée; Groupe 2: les rats ont subi une occlusion permanente de la carotide gauche et ont reçu un placebo; Groupe 3: les rats ont reçu de la glycine i.p. (40 mMol kg^{-1} 24 h^{-1}) pendant 30 jours après l'occlusion carotidienne. Trente jours après la chirurgie les animaux ont été sacrifiés et les cerveaux examinés par histopathologie. **Résultats:** Groupe 1: le cerveau a une histopathologie normale. Groupe 2: présence de vaisseaux capillaires cérébraux tortueux ainsi que de zones de nécrose dans l'hippocampe, le noyau vestibulaire médian, les cortex piriforme et cérébral, et le thalamus. Groupe 3 : les altérations sont limitées au thalamus. **Conclusions:** La glycine administrée en i.p. chez des rats avec occlusion permanente de la carotide gauche limite les lésions ischémiques cérébrales probablement en augmentant sa disponibilité neurologique en suffisance pour prévenir la

désensibilisation des récepteurs au NMDA et altérer la cascade qui aboutit à la mort cellulaire.

Mots clés

Ischémie cérébrale; Glycine; Agents neuroprotecteurs

RESUMEN

Objetivo: Evaluar los efectos neuroprotectores de la glicina en un modelo animal de daño cerebral por isquemia permanente. La glicina es un aminoácido neurotransmisor inhibitorio que actúa como modulador del receptor N-metil-D-aspartato receptor (NMDA), implicado en forma importante en el proceso de isquemia cerebral. **Métodos:** 24 ratas Wistar de 20 días de edad fueron asignadas aleatoriamente a uno de tres grupos. En el Grupo 1 las ratas fueron sometidas a una cirugía falsa donde la carótida izquierda fue visualizada; en el Grupo 2 se realizó una oclusión permanente de la arteria carótida izquierda y los animales recibieron placebo, y en el Grupo 3 las ratas recibieron glicina i.p. (40 mMol kg^{-1} 24 h^{-1}) durante 30 días posteriores a la cirugía. A los 30 días posteriores a la cirugía, las ratas fueron sacrificadas y sus cerebros analizados por histopatología. **Resultados:** En el Grupo 1 se observó una histopatología normal del cerebro; en el Grupo 2 se observaron vasos capilares tortuosos además de áreas necróticas en el hipocampo, núcleo medio vestibular, la corteza periforme y cerebral, y tálamo. En el Grupo 3, las modificaciones histopatologías fueron restringidas al tálamo. **Conclusiones:** La administración de glicina i.p. a ratas con oclusión permanente de la arteria carótida izquierda limitó el daño secundario a isquemia cerebral, probablemente debido al incremento de la concentración neurológica de glicina a niveles suficientes como para prevenir la desensibilización de los receptores de NMDA, alterando la cascada de acontecimientos que llevan a la muerte celular.

Palabras clave

Isquemia cerebral; Glicina; Agentes neuroprotectores

INTRODUCTION

Ischemia of central nervous system is associated with increased extracellular concentrations of glutamate, which leads to uncontrolled activation of NMDA receptors and favors the influx of calcium. These changes progress to mitochondrial and DNA damage, ion imbalance, cell membrane disruption and, finally, cell death. As death cells release glutamate, this process can be extended to neighboring cells [1].

Several experimental studies have shown that inhibition of glutamate release may offer a neuroprotective alternative [2] [3]. Glycine is an inhibitory neurotransmitter in the brain and spinal cord, and plays a critical role as a modulator of NMDA receptors [4]. Furthermore, peripheral administration of glycine produces a clear dose-dependent elevation of the levels of this amino acid in both serum and cerebrospinal fluid (CSF) [5]. However, the effect of extracellular glycine concentrations on ischemic injury remains controversial as there is evidence that high levels of extracellular glycine are indeed associated to ischemic damage [4] [6].

In order to evaluate the neuroprotective effects of glycine in an experimental animal model of brain ischemia, we assessed the histopathology of brains obtained from rats treated for 30 days after unilateral carotid occlusion.

METHODS

Animals

The study was approved by the Institutional Committee for Animal Use at the National Institute of Rehabilitation, and rats were handled according to the national guidelines in Mexico. Twenty-four Wistar rats, weighing approximately 25 g at the beginning of the study, were randomly allocated to three groups (n= 8 animals/group). Animals in Group 1 underwent a sham operation where the common carotid artery (CCA) was isolated but not occluded. Occlusion of the CCA was performed to animals in Groups 2 and 3. Rats were maintained on a 12 hour light/dark cycle with unlimited access to food and water. Glycine (Sigma-Aldrich Quimica SA de CV, Toluca, Estado de México, Mexico) was dissolved in 0.9% normal saline and administered intraperitoneally (i.p.) to animals in Group 3 at a dose of 40 mMol kg^{-1} day^{-1} for 30 days post-ligation whereas the animals in Groups 1 and 2 received saline at a volume adjusted to simulate treatment with glycine.

Surgical procedure

The morning of the surgery, animals were anesthetized with i.p. administration of ketamine (50 mg kg^{-1}) and xylazine (5 mg kg^{-1}). The animals were placed in dorsal position and their extremities were fixed; the skin was clamped and exposed by a gentle traction of the clamps; an incision of approximately 2.5 cm long was performed in the mean anterior line of the neck, at approximately 3 cm distal to the sternum. Using the omohyoid and sternocleidomastoid muscles as references, we located and dissected the vascular package. We identified the internal jugular vein, the CCA (pulsating), and the vague nerve. The OCC was dissected, occluded with silk suture and sectioned. For the purpose of standardizing the surgical procedure, occlusion was always performed to the left CCA. The sectioned segment was analyzed histopathologically in order to confirm that the proper vessel was occluded. The sham operation was undertaken in a similar manner, except CCA were not occluded or sectioned.

Histological evaluation

Thirty days after the surgical procedure, animals were anesthetized as described above. A transcardiac perfusion through the left ventricle was performed with formaldehyde 4% in a buffer of phosphates (pH 7.0). The brain was extracted and maintained in this solution for a period not longer than a week. Thereafter, brains were embedded in paraffin and cut in 5-µm sections. Cresyl violet staining was used for the assessment of cell loss. A total of 45 slices were prepared per brain.

RESULTS

As expected, in Group 1 (sham operation and treatment with saline) the cerebral cortex, hippocampus (Figure 1 A & D), thalamus and cerebellum (Figure 2 A, D & G) showed normal morphology. In Group 2 (occlusion of CCA and treatment with saline), the hemisphere contralateral to the occlusion showed histopathological damage including capillary vessel dilation in all brain regions. There were some hyperchromic cells in the cortex (Figure 1) and dentate gyrus. In the hippocampus, we observed areas of dilated capillaries with endothelium damage and necrotic granular cells. The thalamus was found with some capillaries dilated and endothelial damage (Figure 2 B, E & H). In Group 3, most animals had brain alterations restricted only to thalamic structures. In the hippocampus, very few dilated capillaries were observed. Thalamus showed only few dilated capillaries with endothelial damage (Figure 2 C). The cerebellum had normal morphology (Figure 2 F & I).

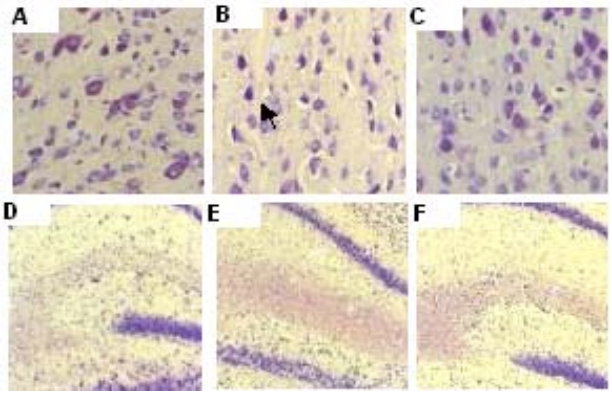

Figure 1. Cerebral cortex (A, B, C; magnification × 200) and hippocampus (D, E, F; magnification × 200) following 30 days of ischemia. Normal morphology was observed in the sham-operation group (A & D). In Group 2 (occlusion-saline), necrotic areas were observed in the cortex (arrow) & hippocampus (B & E). In Group 3 (occlusion-glycine), hyperchromic cells were found in cortex and dentate gyrus, hippocampus with dilated capillaries and damage in endothelium (C & F). Slices were stained with cresyl violet.

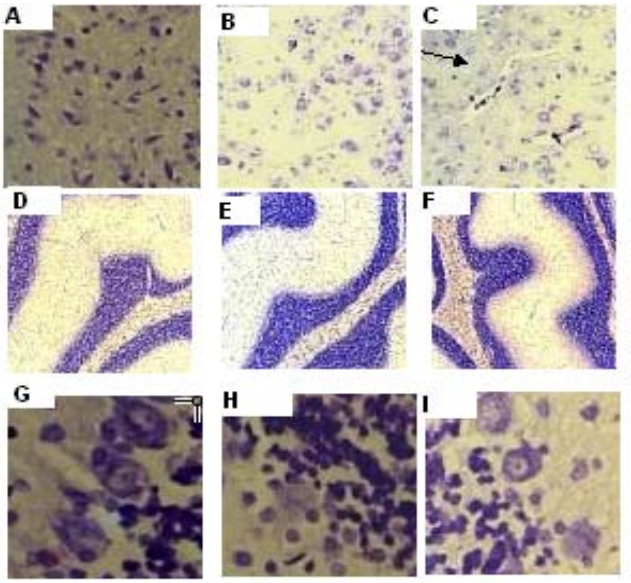

Figure 2. Thalamus (A, B, C; ; magnification × 200) and cerebellum (D, E & F at magnification × 100; and G, H & I at magnification × 200) of rats after 30 days of ischemia. Normal cellular pattern was observed in Group 1 - sham operation (A, D & G). In Group 2, groups of necrotic neurons were found in the thalamus and cerebellum (B, E & H). In the glycine-treated group, only dilated capillaries and some level of capillary endothelial damage (arrow) were observed in thalamus whereas no alterations were appreciated in the cerebellum (F & I).

DISCUSSION

The present study found that administration of glycine in an animal model of brain ischemic injury prevented cell damage. Glycine is normally found in the interstitial space at a brain concentration of 4 mol L^{-1} in the cortex [7] [8], and is a well-recognized major inhibitory neurotransmitter in the spinal cord as well as playing an important role as a co-agonist of NMDA subtype receptors [9] [10]. NMDA receptor has been implicated in a number of processes related to neuronal development and plasticity as well as in neuronal survival [11] [12]. Accordingly, it was previously found that exogenously applied glycine facilitates the generation of long time potentiation (LTP) in CA1 and dentate region of rat hippocampal slices by activating glycine modulatory sites associated with NMDA receptors [10] [13]. The pyramidal neurons of hippocampus sector CA1 are considered as the most vulnerable neurons to ischemia [14] [15]. Furthermore, injection of glycine and AFK 90-7 (a glycine phosphorylated derivative) increased the survival of laboratory animals under the conditions of ischemia caused by ligation of carotid artery [16]. Administration of peripheral glycine produced a distinct rise in levels of glycine in serum and cerebrospinal fluid [5]. However, levels of glycine have been associated with

the severity of hypoxic encephalopathy-ischemic observed in human infants [17]. In addition, rats have a large capacity to recover, and in many behavior tests the impairment in performance after focal cerebral ischemia is transient [18]. Therefore, more studies are needed in order to confirm the protective effects observed in our study. In this context, the model of ischemia with permanent occlusion of CCA would be preferable over models of transient ischemia for testing drug treatment of brain ischemic damage.

CONCLUSION

Glycine administered i.p. to rats with permanent left carotid occlusion limited ischemic brain damage, probably by increasing the neurological availability of glycine concentration enough to prevent the desensitization of NMDA receptors and consequently altering the cascade of events that lead to cellular death.

AUTHORS' PARTICIPATION

R U-E, E A-T and A A-R conceived the study and performed the experiments. All the authors participated discussing the results and drafting the manuscript.

CONFLICT OF INTERESTS/DISCLAIMERS

AA N-O is member of the Editorial Board of the journal.

REFERENCES

[1] Mokudai T, Ayoub IA, Sakakibara Y, Jian Lee E, Ogilvy CS, Maynard KI. Delayed treatment with nicotinamide (Vitamin B3) improves neurological outcome and reduces infarct volume after transient focal cerebral ischemia in Wistar rats. Stroke 2000; 31: 1679–1685.

[2] Lipton SA, Rosenberg PA. Excitatory acids as a final common pathway for neurologic disorders. N Engl J Med 1994; 330: 613–622.

[3] Nava-Ocampo AA, Reyes-Pérez H, Bello-Ramírez AM, Mansilla-Olivares A, Ponce-Monter H. For ischemic brain damage, is preclinical evidence of neuroprotection by presynaptic blockade of glutamate release enough? Med Hypotheses 2000; 54: 77-79.

[4] Oda M, Kure S, Sugara T, Yamaguchi S, Kojima K, Shinka T, et al. Direct correlation between ischemic injury and extracellular glycine concentration in mice with genetically altered activities of the glycine cleavage multienzyme system. Stroke 2007; 38: 2157-2164.

[5] D'Souza DC, Gil R, Cassello K, Morrissey K, Abi-Saab D, White J, et al. IV glycine and oral D-cycloserine effects on plasma and CSF aminoacids in healthy humans. Biol Psychiatry 2000; 47: 450–462.

[6] Katsuki H, Watanabe Y, Fujimoto S, Kume T Akaike A. Contribution of endogenous glycine and d-serine to excitotoxic and ischemic cell death in rat cerebrocortical slice cultures. Life Sci 2007; 81: 740-749.

[7] Shimizu-Sasamata M, Bosque-Hamilton P, Huang PL, Moskowitz MA, Lo EH. Attenuated neurotransmitter release and spreading depression-like depolarizations after focal ischemia in mutant mice with disrupted type I nitric oxide synthase gene. J Neurosci 1998; 18: 9564–9571.

[8] Wood PL. The co-agonist concept: is the NMDA-associated glycine receptor saturated in vivo? Life Sci 1995; 57: 301–310.

[9] Jhonson JW, Ascher P. Glycine potentials the NMDA response in cultured mouse. Nature 1987; 325: 529-531.

[10] Szyndler J, Piechal A, Blecharz-Klin K, Skorzewska A, Maciejak P, Wallkowiak J, et al. Effect of kindled seizures on rat behavior in water Morris maze test and aminoacid concentrations in brain structures. Pharmacol Rep 2006; 58: 75-82.

[11] Constatine-Paton M, Cline HT, Debski E. Patterned activity, synaptic convergence, and the NMDA receptor in developing visual pathways. Annu Rev Neurosci 1990; 12: 129-154.

[12] Bliss TV, Collinridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993; 361: 31-39.

[13] Watanabe Y, Saito H, Abe K. Effects of glycine and Structurally related aminoacids on generation of long-term potentiation in rat hippocampal slices. Eur J Pharmacol 1992; 223: 179-184.

[14] Pulsinelli WA, Waldman S, Rawlinson D, Plum F. Moderate hyperglycemia augments ischemic brain damage: a neuropathologic study in the rat. Neurology 1982; 32: 1239-1246.

[15] Smith ML, Bendek G, Dahlgren N, Rosen I, Wieloch T, Siesjö BK. Models for studying long-term recovery following forebrain ischemia in the rat. 2. A 2-vessel occlusion model. Acta Neurol Scand 1984; 69: 385-401.

[16] Makarova LM, Pogorelyi VE. Experimental evaluation of the effect of glycine and its phosphorylated derivative on ischemic brain injury [in Russian]. Eksp Klin Farmakol 2006; 69: 24-26.

[17] Malik GK, Pandey M, Kumar R, Chawla S, Rathi B, Gupta RK. MR imaging and in vivo proton spectroscopy of the brain in neonates with hypoxic ischemic encephalopathy. Eur J Radiol 2002; 43: 6–13.

[18] Corbett D, Nurse S. The problem of assessing effective neuroprotection in experimental cerebral ischemia. Prog Neurobiol 1998; 54: 531-548.