1735-2657/07/61-51-54
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS

IJPT 6:51-54, 2007

RESEARCH ARTICLE

Nimodipine Protects PC12 Cells against Oxygen-Glucose Deprivation

NAHID RAHBAR-ROSHANDEL, LILI RAZAVI and BAHAREH TAVAKOLI-FAR

For author affiliations, see end of text.

Received July 13, 2006; Revised July 29, 2007; Accepted July 30, 2007

This paper is available online at *http://ijpt.iums.ac.ir*

ABSTRACT

The protective effect of an L-type calcium channel blocker, nimodipine, on cell injury induced by oxygen-glucose deprivation (OGD) in PC12 cells was investigated. PC12 cells were exposed to oxygen-glucose deprivation (30 minutes and 60 minutes respectively) in the presence or absence of nimodipine (10μM/L) in three different time schedules (pre-24h, pre-3h and concurrently). Cellular viability was assessed by MTT assay. OGD-induced cell injury was significantly attenuated by nimodipine in all three treatment schedules. Application of MK801 (10μM/L), an antagonist of NMDA glutamate receptors also inhibited PC12 cell death induced by OGD. Our study suggests that nimodipine has protective effects against OGD-induced neurotoxicity..

Keywords: *Nimodipine, Oxygen-glucose deprivation, PC12 cell line*

Brain requires a continuous supply of oxygen and glucose to maintain normal functions. Loss of this supply, even for a short period, leads to irreversible brain injury, including neuron degeneration and cell death. The primary cause of the vulnerability of neurons may be related to the fact that oxygen-glucose deprivation (OGD) is mediated by deregulation of extracellular levels of glutamate. Glutamate is a principal excitatory neurotransmitter. Exposure of the neurons to high concentration of glutamate that occurs during ischemia and a variety of pathologic conditions like stroke and various neurodegenerative disorders such as Alzheimer's disease can lead to neuronal death [1].

The destructive effects of excess glutamate are mediated by glutamate receptors, particularly those of the NMDA type. Activated NMDA receptors elevate calcium influx and open voltage-gated channels [2]. A variety of studies have led to the hypothesis that Ca2+ surplus appears to play a critical role in the genesis of neuronal injury followed by activation of protein kinases, phospholipases, nitric oxide synthesis, impaired mitochondrial functions, the generation of free radicals and finally leading of this sequence to neurodegeneration and cell death [3].

Various classes of calcium-channel blockers have been introduced [4]. Among these classes, dihydropyridines (DHP_s) derivatives are widely used to show the existence of L-type class of ca^{2+} channels. Nimodipine has been shown to dilate cerebral arterioles and to increase cerebral blood flow in animals and humans. It is used in the treatment of a range of cerebrovascular disorders [5]. Major interests to date, however, have focused on its use in the prevention and treatment of the delayed ischemic neurological deficits [6]. But the effectiveness of nimodipine is still unknown. Despite some positive reports about it, some studies indicate that it is ineffective in ischemic insults [7] and some report that nimodipine worsens neuronal degeneration and can induce neuronal cell death when compared with placebo [8].

In the current study, we examined the effects of nimodipine on OGD-induced neurotoxicity, in PC12 cells, a rat pheochromacytoma cell line.

MATERIALS AND METHODS

Materials

Nimodipine was supplied from Bayer and dissolved in methanol at a concentration of 1mM as a stock solution. The methanolic solution was further diluted with DMEM to obtain desired concentrations. The highest concentration of methanol in each well of the plates was lower than 0.1%; this had no significant cytotoxic ef-

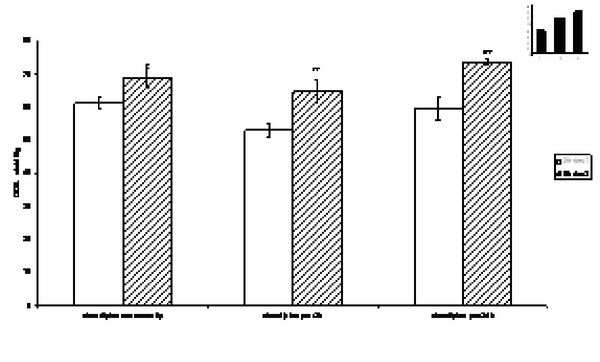

Fig 1. The effects of nimodipine on three different time schedules, concurrently, pre-3 h and pre-24 h under OGD conditions (30min.) on PC12 cell culture. The difference between the effects of nimodipine and control group is platted in the inset. * $p < 0.05$, ** $p< 0.005$, *** $p< 0.0005$

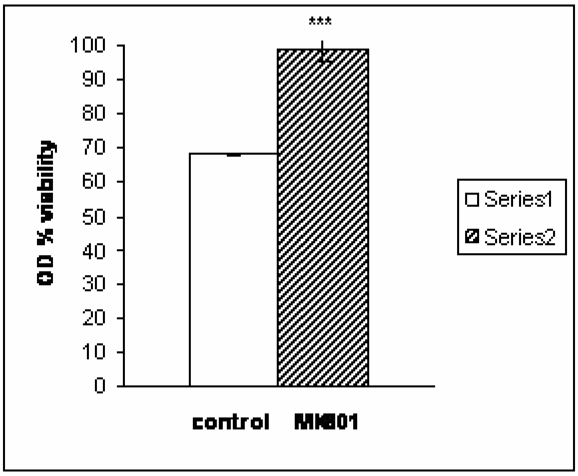

Fig 2. The effects of NMDA glutamate receptor antagonist, (MK-801), on OGD-induced cell injury. *** $p< 0.0005$

fects on PC12 cells. Methyl tetrazolium bromide (MTT) was purchased from sigma

Cell line

PC12 cells were obtained from Pasteur Institute of Iran (Tehran / Iran) and were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO BRL), supplemented with 10% fetal bovine serum (FBS, heat-inactivated), 5% horse serum (HS, heat-inactivated) from (GIBCO BRL), 100 IU /ml penicillin and 100 μg/ml streptomycin (GIBCO BRL), in a humidified incubator aerated with 5% CO2 in air at 37˚C. The cells were subcultured twice a week by gentle scraping and cultured at a density of 6-8×105cells/cm2 in 96-well plates. Cells were used for experiments 24 h after seeding.

Viability measurements

The cytotoxic effect was assessed using a tetrazolium-based colorimetric assay (MTT assay) [9]. The cells were cultured into a 96-well flat-bottomed tissue culture plate. After each treatment, MTT (5mgr/ML) was added to each culture well. After incubation at 37˚C for 3 hours, the formazan crystals were dissolved by addition of 100μL dimethyl soulfoxide (DMSO), and the plates were shaken vigorously to ensure complete solubilization. Formazan absorbance was assessed at 570 nm by a microplate reader (Dynex MMx). Values were expressed as percentage of viable cells.

Drug administration schedules for Oxygen-glucose deprivation

Procedures for OGD were performed as described previously [10]. Briefly, cell cultures were treated with nimodipine with three time schedules (24 h, 3 h and concurrently). Afterwards, the culture medium was replaced with glucose-free Krebs buffer (KR) with the following ionic composition: 5.36 mM KCL, 1.26 mM CaCl2, 0.44 mM KH2PO4, 0.49 mM MgCl2, 0.41 mM MgSO4, 137 mM NaCl, 4.17 mM NahCO3, 0.34 mM NaHPO4, and 10 mM HEPES (pH 7.4) and cultures were exposed to hypoxia for 30 and 60 minutes in a small anaerobic jar previously filled with 95% (v/v) N2 and 5% (v/v) CO2 at 37oC. The OGD was ended by replacing the KR buffer with DMEM and the cells were returned to incubator with 95% atmosphere and 5% CO2 for additional 24h. Citotoxicity was quantified by MTT assay and values were expressed as percentage of viable cells.

Data analysis

Data were expressed as means ± SEM. The significance of differences between means was determined with student's t-test. * $p < 0.05$, ** $p< 0.005$, *** $p< 0.0005$.

RESULTS

The effects of nimodipine on OGD-induced cell injury on PC12 cell line

In order to test the effects of nimodipine against OGD-induced cell injury, we exposed PC12 cells to OGD for 30 and 60 minutes. As shown in Fig.1, after 30 minutes OGD the drug significantly decreased OGD-induced cell injury on three different treatment schedules (concurrently, pre-3 h and pre-24 h). The drug on the pre-24 hour and pre-3 hour schedules dramatically increased cell viability. In concurrent treatment schedule nimodipine significantly but partially decreased OGD-induced cell injury. In contrast, after 60 minutes insult nimodipine could not protect OGD-induced cell injury (data not shown).

OGD-induced NMDA receptor- dependent cell injury

We examined the blockade of NMDA glutamate receptor to determine if glutamate contributed to ischemic injury. MK-801 (10μM) an antagonist at NMDA subtype of glutamate receptors, markedly attenuated OGD-induced cell injury (Fig.2). This result suggested that glutamate release and subsequent NMDA receptor activation is the major cause of OGD-induced cell injury.

DISCUSSION

L-type voltage-dependent calcium-channel blockers, have been reported to protect the neurons against glutamate-induced toxicity and OGD-induced cell injury [11, 12]. We investigated whether the calcium-channel blocker, nimodipine can protect PC12 cells against ischemic insult. In this work, we used PC12 cells, a rat pheochromacytoma cell line, as a model of neurons. OGD was employed as an in-vitro ischemic model. The results obtained in this study showed that nimodipine could effectively protect the PC12 cells against cell death induced by OGD. These effects were similar to those obtained with nimodipine effects in glutamate induced-cell death [12].

We observed that when cells were exposed to 30 minutes OGD, the drug dramatically suppressed the PC12 cells death. It could be suggested that voltage dependent calcium channels play a role in extracellular glutamate receptor activation. Glutamate is an important excitatory amino acid working at a variety of excitatory synapses in nervous system. It causes important impression on fundamental cellular actions like synaptic plasticity, neuronal development and excitation via the activation of glutamate receptors [13, 14]. Compelling evidence have led to the fact that excess extracellular glutamate concentration leads to cell loss in in-vitro studies using cultured neurons [15, 16]. On the other hand, substantial evidence indicates that OGD is accompanied by massive increases in extracellular glutamate, which plays a crucial role in induction of neuronal cell injury [17]. Notably MK-801, an antagonist of glutamate at NMDA receptors markedly attenuated OGD-induced cell toxicity. In other words, NMDA receptor activation is the major cause of OGD-induced cell injury. In addition, nimodipine did not show a significant effect on cell death when OGD was applied for 60 minute, suggesting that involvement of excytotoxic release of neuronal glutamate is restricted to an early stage of ischemia. The exact mechanisms by which intra cellular Ca2+ concentration are increased during ischemia is still not completely understood but the glutamate-induced Ca2+ overload hypothesis has been widely accepted as the mechanism of neuronal injury in glutamate-induced excitotoxicity and in injury occurring in cerebral hypoxia or ischemia [18, 2, 19, 20]. L-type calcium channels which are present throughout the central nervous system have an important role in excitatory process. Our current results show that nimodipine can significantly decrease cell injury induced by OGD in PC12 cells; this may be related to blockade of L-type calcium channels and attenuation influx of calcium into the PC12 cells. In differentiated PC12 cells, MK-801 was tested and showed that it is capable of blocking NMDA receptors and inhibiting excess glutamate release [21, 22].

In conclusion, our results showed that nimodipine, a 1, 4- dihydropyridines, belonging to class III calcium channel antagonists could protect PC12 cells against neurotoxicity induced by OGD.

ACKNOWLEDGEMENT

This work was supported by Razi Institute for Drug Research, Iran University of Medical Sciences, Tehran, Iran. The authors wish to thank Dr. S.A. Ebrahimi for his assistance in the data analysis and Dr. P. Rahimi-Moghaddam for reading this manuscript.

REFERENCES

1. Olnay JW.Excitotoxic amino acids and neuropsychiatric disorder. Annu. Rev. Pharmacol.Toxical. 1990; 30: 47-71.
2. Choi DW,Rothman SM.The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. Annual Review of Neuroscience 1990;13:171-182.
3. Beckman JS The double-edged role of nitric oxide in brain function and superoxide mediated injury. J. Dev. Physiol. 1991;15:53-59.
4. Flekenstien A. Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. Ann. Rev. Pharmacol.Toxic.1977;17:149-166.
5. Blardi P, Urso R, de Lalla A, Volpi L, Di Perri T, Auteri A. Nimodipine;Drug pharmacokinetics and plasma adenosine levels in patients affected by cerebral ischemia. Clin. Pharmacol. Ther. 2002;72:551-566.
6. Dreir JP, Windmuller O, Petzold G, Lindauer U, Einhaupl KM, Dirnaugl U..Ischemic triggerd by red blood cell products in the subarachnoid space in ihibited by nimpodipine administration or moderate volume expansion/hemodilution in rats. Neurosurgery 2002;51:1457-1465.
7. Rinkel GJ, Feigin VL, Algra A, Vermeulen M, van Gijn J. Calcium antagonists for aneurismal subarachnoid haemorrhage cochrane. Database Syst. Rev. 2002;4: CD000277.
8. Trust Study Group. Randimized, double blind, placebo controlled trial if nimodipine I acute stroke. Lance . 1990;336:1205-1209.
9. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods . 1983;65: 55-63.
10. Frantsevan MV, Carlen PL, El-Beheiry, H.A submersion method to induce hypoxic damage in organotypic hippocampal cultures. J. Neurosci. Methods 1999;89: 25-31.
11. Kornekov AL, Pahnake J, Frei K, Warzok R, Schroder HW, Frick R, Muljana L, Piek J, Yonekawa Y. Treatment with nimodipine and mannitol reduces programed cell and infarct size following focal cerebral ischemia. Neurosurg.Rev.2000; 23: 145-150.
12. Gepdiremen A, Duzenli S, Hacimuftuoglu A, Suleyman H, Oztas S.The effects of dantroline alone or in combination with nimodipine in glutamate-induced neurotoxicity in cerebellar granular cell cultures of rat pups. Pharmacological Research 2000;43: 241-244.

13. Bleich S, Romer K, Wiltfang J, Kornhuber J. Glutamate and the glutamate receptor system: a target for drug action.International Journal of Geriatric Psychiatry . 2003;18: S33-S40.

14. Conn PJ.Physiological roles and therapeutic potential of metabotropic glutamate receptors. Annals of the New York Academy of Science 2003;1003: 12-21.

15. Choi DW, .Ionic dependence of glutamate neurotoxicity. Journal of Neuroscience 1987;7: 369-379.

16. Ankarcrona M, Dypbukt JM, Bonfoco E, Zhivotovsky B, Orrenius S, Lipton SA, Nicotera P. Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function. Neuron 1995;15: 961-973.

17. Rothman SM, Olney JW.Glutamate and pathophysiology of hypoxic-ischemic brain damage. Ann. Neurol. 1986;19: 105-111.

18. SilverI A, Erecinska M.Intracellular and extracellular changes of [ca^{2+}] in hypoxia and ischemia in rat brain in vivo. J. Gen. Physiol. 1990;5: 837-866.

19. Kritian T, Siesjo BK Calcium-related damage in ischemia. Life Sciences 1996;59: 357-367.

20. Jabaudon D, Scanziani M, Gahwiler BH, Gerber U.Acute decrease in net glutamate uptake during energy deprivation.Proc.Natl.Acad. Sci.U.S.A 2000;97:5610-5615.

21. NishizawaY.Glutamate release and neuronal damage in ischemia. Life Sci.2001; 69: 369-381.

22. Benvenga MJ, Spaulding TC.Amnesic effect of the novel anticonvulsant MK-801. Pharmacology Biochemistry and Behavior 1988;30: 205-207.

CURRENT AUTHOR ADDRESSES

Nahid Rahbar Roshandel, Razi Institute for Drug Research (RIDR), Iran University of Medical Sciences, Tehran, Iran. E-mail: nahid@iums.ac.ir (Corresponding author)

Lili Razavi, Department of Biology, School of science, North Tehran Branch , Islamic Azad University, Tehran, Iran.

Bahareh Tavakoli-Far, Razi Institute of Drug Research, Iran University of Medical Science, Tehran, Iran.