Effect of Subchronic 3-Monochloro-1,2-propanediol Exposure on the Expression of Inducible Nitric Oxide Synthase in Rat Brain

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Abstract

3-Monochloro-1,2-propanediol (3-MCPD) is a contaminant of acid-hydrolyzed vegetable protein. Several reports have suggested that chronic exposure to 3-MCPD could produce neurotoxicity *in vitro* or neurobehavioral effects inaspects of experimental animals. Disturbance of the nitric oxide signaling pathway by chronic exposure to 3-MCPD may be a causal factor of neurological disorders in rats. In order to investigate the relationship between 3-MCPD administration and expression of inducibal nitric oxide synthase (iNOS), the numbers and distribution patterns of iNOS-immunoreactive neurons were examined. At the all three bregma level examined, the optical density of iNOS-postive neurons was significantly increased following exposure to 3-MCPD. The change was more severe in the upper layer than in deep layer of the cortex. These data suggest that 3-MCPD toxicity may be mediated through disturbances to the nitric oxide signaling pathway.

Introduction

3-Monochloro-1,2-propanediol (3-MCPD) is a contaminant of hydrolyzed vegetable protein foodstuffs, including acid-hydrolyzed soy sauce. In addition, 3-MCPD can be formed in some foods during cooking processes. Even now, the general population in many countries is consuming a considerable amount of 3-MCPD from contaminated soy sauces or other food ingredients. Many studies using experimental animals have shown that 3-MCPD causes a variety of toxicological changes in blood, kidneys, testes, and sperm. On the other hand, other toxicological studies have demonstrated that 3-MCPD produced neurotoxic changes in astrocytes and induced severe fore- and hind-limbs paralysis in mice at single doses of 90 mg/kg body weight. Nitric oxide (NO) is known to play a numerous role in the brain, including activation of soluble guanylate cyclase and modulation of synaptic vesicle exocytosis. The present study was undertaken in order to determine whether prolonged exposure to 3-MCPD could change the expression of inducibal nitric oxide synthase (iNOS), which is the key enzyme in the production of NO, in the frontal cortex and striatum of rat. Using immunohistochemical method, we

measured iNOS expression in the frontal sections of rats. We looked at differences in iNOS expression between cortical and striatal areas, with the treatment of 3-MCPD. This allowed us to assess which of these two cortical zones might have the greatest potential for neurotoxicity.

Materials and Method

The SD rats in the control and 3-MCPD groups were given saline or 3-MCPD (Sigma, St. Louis, MO) at doses of 30 mg/kg body weight (p.o.) on a daily basis for 13 weeks. After perfusion, sections of 40 um were cut on a freezing microtome. Immunocytochemistry was performed on free-floating sections with anti-iNOS antiserum (Transduction Laboratories, Lexington, KY). After sections were mounted on gelatin-coated slides, The optical density of iNOS-positive neurons was counted in one hemisphere, at bregma 1.00 level according to the Paxinos atlas. All the neocortical areas and randomly selected four striatal areas were examined under light microscopy at a magnification of X200. To examine the pattern of distribution of iNOS-immunoreactive neurons throughout layers of cortex, coronal sections from each animal were charted using a research microscope fitted with a drawing tube. Statistical analysis of iNOS positive neurons were conducted using the two-tailed Student's t-test where P values < 0.05 were considered significant.

Results and Discussion

Overall, iNOS-immunoreactive neurons were well represented in all of the studied areas and were scattered throughout the cortex and the striatum. The optical densities of the iNOS-positive neurons in the cingulated, motor, and primary somatosensory cortices of 3-MCPD treated rats were higher than in the control. Especially, the motor and somatosensory cortices showed statistically significant changes after treatment with 3-MCPD ($p<0.05$ and $p<0.01$, respectively). In contrast, the number of iNOS-positive neurons in the insular cortex did not change significantly after treatment with 3-MCPD. Total optical density of iNOS-positive neurons in cortex was found to be significantly lower (17.9% less) following exposure to 3-MCPD ($p<0.05$). In contrast, in the striatum, the change in the optical density of iNOS-positive neurons with 3-MCPD treatment did not significantly differ from that of control.

Conclusion

The findings reported suggest that subchronic 3-MCPD exposure-induced alterations in NOS expression may reflect changes in the molecular signal transduction of the gluatamate-NO-cGMP pathway in the neocortex and the striatum, especially in the caudal brain area. In addition, these results suggest that the neurotoxic effects of 3-MCPD might be relevant to a NO homeostasis disturbance through the differential expression of iNOS throughout the rostrocaudal extent. Besides, the results indicate that the neurotoxic effects of 3-MCPD might be relevant to a disturbance of the global recruiting action response and synchronization which are responsible for wide networks of cortical activity in the frontal cortex.

References

Meffert M.K., Premack B.A. and Schulman H. : Nitric oxide stimulates Ca(2+)-independent synaptic vesicle release. Neuron, 12(6), 1235-1244, 1994.

Wildemann B. and Bicker G. : Nitric oxide and cyclic GMP induce vesicle release at Drosophila neuromuscular junction. J. Neurobiol, 39(3), 337-346, 1999.

Boehning D. and Snyder S.H. : Novel neural modulators. Annu. Rev. Neurosci, 26, 105-131, 2003.

Seyidova D., Aliyev A., Rzayev N., Obrenovich M., Lamb B.T., Smith M.A., de la Torre J.C., Perry G. and Aliev G. : The role of nitric oxide in the pathogenesis of brain lesions during the development of Alzheimer's disease. In Vivo, 18(3), 325-333, 2004.

Knowles R.G. and Moncada S. : Nitric oxide synthases in mammals. Biochem. J., 298, 249-258, 1994.

Bredt D S, Glatt C E, Hwang P M, Fotuhi M., Dawson T M. and Snyder S.H · Nitric oxide synthase protein and mRNA are discretely localized in neuronal populations of the mammalian CNS together with NADPH diaphorase. Neuron, 7(4), 615-624, 1991.

Yuan T., Vogel H.J., Sutherland C. and Walsh M P. : Characterization of the Ca^{2+} -dependent and -independent interactions between calmodulin and its binding domain of inducible nitric oxide synthase FEBS Lett, 431(2), 210-214, 1998.

Griffith O W. and Stuehr D J. : Nitric oxide synthases: properties and catalytic mechanism. Annu. Rev. Physiol, 57, 707-736, 1995

Iadecola C., Zhang F., Casey R., Clark H.B. and Ross M.E. : Inducible nitric oxide synthase gene expression in vascular cells after transient focal cerebral ischemia. Stroke. 27(8), 1373-1380, 1996.

Heneka M.T. and Feinstein D.L. : Expression and function of inducible nitric oxide synthase in neurons. J. Neuroimmunol., 114(1-2), 8-18, 2001.

Nathan C. and Xie Q.W. : Regulation of biosynthesis of nitric oxide. J. Biol. Chem., 269(19), 13725-13728, 1994.

Beckman J.S. : The double-edged role of nitric oxide in brain function and superoxide-mediated injury. J. Dev. Physiol., 15(1), 53-59, 1991.

Garthwaite J. : Glutamate, nitric oxide and cell-cell signalling in the nervous system. Trends Neurosci., 14(2), 60-67, 1991.

Meffert M.K., Calakos N.C., Scheller R.H. and Schulman H. : Nitric oxide modulates synaptic vesicle docking fusion reactions. Neuron, 16(6), 1229-1236, 1996.

Kiss J.P. and Vizi E.S. : Nitric oxide: a novel link between synaptic and nonsynaptic transmission. Trends Neurosci, 24, 211-215, 2001.

Crews C., Brereton P. and Davies A. : The effects of domestic cooking on the levels of 3-monochloropropanediol in foods. Food Addit Contam, 18, 271-280, 2001.

Crews C., Hough P., Brereton P., Harvey D., Macarthur R. and Matthews W. : Survey of 3-monochloropropane-1,2-diol (3-MCPD) in selected food groups, 1999-2000. Food Addit Contam, 19, 22-27, 2002