Available online at www.sciencedirect.com
ScienceDirect
Toxicology 239 (2007) 136–143
TOXICOLOGY
www.elsevier.com/locate/toxicol

Role of nitric oxide system in hydroxyl radical generation in rat striatum due to carbon monoxide poisoning, as determined by microdialysis

Shuichi Hara [a,*], Toshiji Mukai [b], Kunihiko Kurosaki [c], Hajime Mizukami [a], Fumi Kuriiwa [a], Takahiko Endo [a]

[a] *Department of Forensic Medicine, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan*
[b] *Department of Legal Medicine, St. Marianna University School of Medicine, Kanagawa 216-8511, Japan*
[c] *Department of Legal Medicine, Toho University School of Medicine, Tokyo 143-8540, Japan*

Received 11 June 2007; received in revised form 7 July 2007; accepted 9 July 2007
Available online 13 July 2007

Abstract

We explored the possible role of the nitric oxide (NO) system in hydroxyl radical (•OH) generation induced by carbon monoxide (CO) poisoning in rat striatum by means of microdialysis with the use of NO synthase (NOS) inhibitors, N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA), as well as L-arginine (L-Arg; the NOS substrate) and D-arginine (D-Arg). The CO-induced •OH generation was suppressed by both L-Arg and D-Arg. It was also suppressed by L-NAME, which inhibits generation of reactive oxygen species (ROS) *via* neuronal NOS (nNOS) and inducible NOS, but not *via* endothelial NOS. In contrast, L-NMMA, which inhibits only ROS generation *via* inducible NOS, potentiated the •OH generation. L-Arg completely reversed the L-NAME effect and partly reversed the L-NMMA effect. D-Arg reversed the L-NAME effect more potently than did L-Arg, resulting in much more •OH generation than was observed with CO alone, and also potentiated the L-NMMA effect. On the other hand, W-7, an antagonist of calmodulin, which is critical for nNOS activity, had no effect on the CO-induced •OH generation. These findings suggest that complex mechanisms operate in •OH generation in rat striatum upon CO poisoning and that the NO system might not be included among those mechanisms.

Keywords: Carbon monoxide; Hydroxyl radical; L-Arginine; D-Arginine; Nitric oxide synthase inhibitors; Rat striatum

1. Introduction

Carbon monoxide (CO) poisoning occurs frequently throughout the world (Raub et al., 2000) and induces neuropsychological symptoms, including amnesia and parkinsonism, accompanied with pathological changes of brain tissues, in humans (Choi and Cheon, 1999; Gale et al., 1999; Ginsberg, 1980; O'Donnell et al., 2000) and experimental animals (Ishimaru et al., 1992; Nabeshima et al., 1991). We found that CO poisoning stimulated tetrodotoxin-sensitive generation of hydroxyl radical (•OH) in rat striatum (Hara et al., 2004a). Such stimulation of •OH generation may lead to neuronal injury, since oxidative stress is one of the important factors associated with neuronal injury due to brain insults, including brain ischemia and trauma (Gilgun-Sherki et al., 2002; Leker and Shohami, 2002; Lewén et al., 2000).

* Corresponding author. Tel.: +81 3 3351 6141x345; fax: +81 3 3353 7672.
E-mail address: s-hara@tokyo-med.ac.jp (S. Hara).

0300-483X/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2007.07.008

The CO-induced increase in extracellular glutamate in the striatum was a candidate factor associated with the •OH generation, since administration of glutamate stimulates the radical generation in rat striatum (Lancelot et al., 1998; Laplanche et al., 2000). However, the CO-induced •OH generation was insensitive to glutamate receptor antagonists (Hara et al., 2004a).

Nitric oxide (NO) is synthesized from L-arginine (L-Arg) in the presence of NADPH, tetrahydrobiopterin (THB) and O_2, with stoichiometric formation of L-citrulline, by NO synthase (NOS), of which there are three isozymes, termed neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) (Bredt, 1999; Wiesinger, 2001). NO participates in various physiological and pathological processes in the central nervous systems (Bredt, 1999; Wiesinger, 2001). It has been proposed that CO formed in the process of heme degradation by heme oxygenase is a gaseous neurotransmitter or modulator, like NO (Barañano et al., 2001). Exogenous as well as endogenous CO may operate by modifying the NO system (Thorup et al., 1999; Thom et al., 2004) or may act through a mechanism independent of the NO system (Wang et al., 1997).

We demonstrated that extracellular levels of oxidative NO products, NO_2^- and NO_3^-, as well as citrulline, were decreased in the striatum of CO-poisoned rats, strongly indicating suppression of NO production in rat striatum by CO poisoning (Hara et al., 2003). Furthermore, CO poisoning decreased extracellular Arg in rat striatum, and administration of L-Arg, but not its enantiomer, D-Arg, partly reversed the suppression of NO production by CO poisoning (Hara et al., 2003). These findings suggested that, if the substrate were supplied, NOS might still be able to synthesize NO even under hypoxic conditions due to CO poisoning. It has been shown that NOS isozymes produce reactive oxygen species (ROS), such as superoxide and H_2O_2, instead of NO, at low concentrations of L-Arg and/or THB (Heinzel et al., 1992; Pou et al., 1992; Vasquez-Vivar et al., 1998; Xia and Zweier, 1997). We hypothesized that CO poisoning reduces available L-Arg for NOS, which facilitates generation of ROS, instead of NO, resulting in •OH generation in rat striatum.

L-Arg and an NOS inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), inhibit superoxide and H_2O_2 production by nNOS and iNOS, but not eNOS (Heinzel et al., 1992; Pou et al., 1992; Vasquez-Vivar et al., 1998; Xia and Zweier, 1997). Another NOS inhibitor, N^G-monomethyl-L-arginine (L-NMMA), inhibits superoxide production by iNOS, but not by either nNOS or eNOS (Heinzel et al., 1992; Pou et al., 1992; Vasquez-Vivar et al., 1998; Xia and Zweier, 1997). These substances are widely used to examine the role of NO produced *via* NOS, and therefore, should be useful tools to explore the role of NOS in •OH generation in the CO-poisoned rat striatum. However, these NOS inhibitors, as well as L-Arg and D-Arg, have the ability to scavenge ROS (Lass et al., 2002; Rehman et al., 1997). Rehman et al. (1997) suggested that it is necessary to examine the combined effects of NOS inhibitors with L- or D-Arg on a phenomenon in order to distinguish their potencies to modify the NO system from those to scavenge ROS. In the present study, we examined our hypothesis by exploring the individual and combined effects of the NOS inhibitors and L- and D-Arg on CO-induced •OH generation, by means of brain microdialysis. In addition, we examined the effect of a calmodulin antagonist, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7), on the •OH generation, since calmodulin is required for the NOS activity (Bredt, 1999; Heinzel et al., 1992; Ohashi et al., 2007; Wiesinger, 2001).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, weighing 235–265 g, were purchased from Charles River, Kanagawa, Japan. They were housed in a facility with controlled temperature (22–24 °C) and humidity (50–60%), on a 12-h/12-h light/dark cycle (lights on between 6:00 and 18:00 h) with free access to food and water for at least 1 week before all experiments. The experimental protocol of this work was approved by the Tokyo Medical University Animal Care Committee and all experiments were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals (the Science and International Affairs Bureau of the Japanese Ministry of Education, Culture, Sports, Science, and Technology).

2.2. Determination of •OH by means of microdialysis

•OH generation was estimated by measuring the extracellular level of 2,3-dihydroxybenzoic acid (2,3-DHBA), which is produced through non-enzymatic hydroxylation of salicylic acid by hydroxyl radicals, according to the protocol of Teismann and Ferger (2000), with modifications (Hara et al., 2004a). Rats were put individually into clear plastic chambers (26.5 cm in diameter, 28.5 cm in height), and a microdialysis probe with a 3-mm-long membrane (Eicom, Kyoto, Japan) was inserted into the striatum (coordinates of the tip of the probe; 0.2 mm AP, 3.0 mm L, 6.5 mm DV) (Paxinos and Watson, 1998) through the guide cannula (Eicom), which had been implanted under pentobarbital anesthesia (50 mg/kg, i.p.) at least 5 days before. The microdialysis probe was perfused with a modified Ringer solution (147 mM NaCl, 3 mM KCl, 1.3 mM