ELSEVIER

Pathophysiology 21 (2014) 309–316

ISP PATHOPHYSIOLOGY

www.elsevier.com/locate/pathophys

Experimental studies of remarkable monoamine releases and neural resistance to the transient ischemia and reperfusion

Kanji Yoshimoto [a,b,*], Akira Namera [b], Yousuke Arima [b], Takahiro Nagao [b], Hiroh Saji [c], Tomokazu Takasaka [c], Takeshi Uemura [c], Yoshihisa Watanabe [d], Shuichi Ueda [e], Masataka Nagao [b]

[a] *Department of Food Sciences and Biotechnology, Faculty of Life Sciences, Hiroshima Institute of Technology, Miyake, Saeki-ku, Hiroshima 731-5193, Japan*
[b] *Department of Forensic Medicine, Institute of Biomedical and Health Sciences, Hiroshima University Faculty of Medicine, Kasumi, Minami-ku, Hiroshima 734-8551, Japan*
[c] *Department of Forensic Medicine, Kyoto Prefectural University of Medicine, Kawaramachi, Kamigyo-ku, Kyoto 602-8566, Japan*
[d] *Department of Basic Geriatrics, Kyoto Prefectural University of Medicine, Kawaramachi, Kamigyo-ku, Kyoto 602-8566, Japan*
[e] *Department of Histology and Neurobiology, Dokkyo University School of Medicine, Mibu, Tochigi 321-0293, Japan*

Received 7 May 2014; received in revised form 21 August 2014; accepted 30 August 2014

Abstract

Introduction: The literature described that neural damage caused by ischemia definitely occurs in brain areas. However, few studies have shown real-time changes of extracellular monoamine levels at the time of transient ischemia. **Methods:** We examined changes in the responses of dopamine (DA) and serotonin (5-HT) release in the nucleus accumbens (ACC) of rats treated with four-vessel occlusion (4VO) in experiment 1. In the second experiment, we investigated the selective neural vulnerabilities among the ACC, lateral hypothalamus (LH), and frontal cortex (FC) of rats treated with 4VO and four days of reperfusion. **Results:** The extracellular levels of DA and 5-HT were remarkably increased 200- and 20-fold upon the 10-min clipping of both common carotid arteries in transient cerebral ischemia, respectively. Each increased monoamine release returned to the baseline levels immediately. The release of DA in the ACC and FC was significantly decreased in the rats treated with the coagulation of bilateral vertebral arteries (2VO), compared with that of sham-operated rats. K^+-induced DA release in the ACC and FC of 4VO-treated rats was increased without alteration of DA content. **Discussion:** Surviving dopaminergic neurons in the ACC and FC showed neural hyperfunction associated with the monoamine release, serotonergic neurons in particular these areas exhibiting functional resistance to the transient ischemic change. **Conclusion:** It is suggested that the remarkable extracellular release of DA and 5-HT was not the cause of the ischemic delayed neural degeneration in each brain area, and that the functions of neurotransmitter release involved remarkable resistance to the transient ischemia.

Keywords: Ischemia; Reperfusion; Dopamine; Serotonin; Four-vessel occlusion

1. Introduction

Global and transient cerebral ischemia, which is mostly due to cardiac arrest in humans, induces disturbances of emotion and consciousness as higher function, dizziness, and headache [1,2]. It is believed to be caused by neuronal damage because the brain is dependent on a continuous supply of glucose and oxygen that is regulated by cerebral blood flow [3]. However, the function of the hypothalamic pituitary system has been observed for some period after brain damage, and nerve cells, especially those in the hypothalamic area, have resistance to anoxia and stasis of the bloodstream [4]. Ischemic neuronal damage depends on the blood flow rate in several neural pathways and the demand and supply of energy in different brain areas.

* Corresponding author at: Department of Food Sciences and Biotechnology, Faculty of Life Sciences, Hiroshima Institute of Technology, Miyake, Saeki-ku, Hiroshima 731-5193, Japan. Tel.: +81 82 921 6124.
E-mail address: k.yoshimoto.ud@it-hiroshima.ac.jp (K. Yoshimoto).

http://dx.doi.org/10.1016/j.pathophys.2014.08.005
0928-4680/

Transient cerebral ischemia induces a loss of brain function, and the brain cannot be revived after 8–10 min of ischemia [5]. The literature described that neural damage caused by ischemia definitely occurs in brain areas after a few days [1,6]. However, few studies have shown real-time changes of extracellular monoamine levels at the time of transient ischemia. Using an in vivo brain microdialysis technique [7], we investigated the causal relationship between the real time changes of monoamine release in the nucleus accumbens (ACC), which plays an important role in reinforcement or abuse [8,9], and other areas, and neural changes 4 days after reperfusion with 10 min clipping of the bilateral common carotid arteries of rats with coagulation of both vertebral arteries.

2. Experiment l: changes in the release of dopamine and serotonin in the nucleus accumbens of rats treated with four-vessel occlusion

2.1. Materials and methods

2.1.1. Animals

Eight- to 10-week-old male Wistar rats (280–320 g) were purchased from CLEA Japan, Inc. (Osaka, Japan). The rats were acclimated to and maintained at 23 °C under a 12-h light/dark cycle. They were housed in standard laboratory cages and had free access to food (CE-2, CLEA Japan, Inc.) and water throughout the experiments. All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the protocols were approved by the Committee for the Care and Use of Laboratory Animals, Kyoto Prefectural University of Medicine, Kyoto, Japan (#21-1).

2.1.2. In vivo brain microdialysis and coagulation of bilateral vertebral arteries

The rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and stereotaxically implanted with a brain microdialysis probe guide cannula. All rats were chronically implanted with a brain microdialysis guide cannula (2.0-mm membrane, CMA Microdialysis AB, Kista, Sweden), which was placed 2 mm above the nucleus accumbens (ACC) with reference to the bregma: the coordinates were anterior +2.2 mm, lateral 1.5 mm, and vertical −6.2 mm from the surface of the skull according to the rat stereotaxic atlas [10]. The guide cannula was secured to the skull with two stainless-steel screw anchors and dental cement. Rectal temperature was controlled at 37 °C during and after surgery via a temperature-regulated heating pad.

The animals were allowed over 4–6 days to recover from the treatment of implantation of the brain microdialysis probe guide cannula into the ACC, and were then treated with coagulation of bilateral vertebral arteries, modifying the original method by Pulsinelli and Brierley [11]. After a vertebral midline cervical incision, bilateral vertebral arteries close to the 6th cervical vertbra before entering into the vertebral bone were permanently coagulated (KN-301B, Natsume Co., Japan).

2.1.3. Sample collection at the clipping in four-vessel occlusions

On the experimental day, the brain microdialysis probe was inserted into the ACC through the microdialysis guide cannula under light halothane anesthesia. After obtaining steady release of DA and 5-HT in the ACC, temporary occlusion on bilateral common carotid arteries was performed by clipping with clamps for these arteries of ischemia. Transient occlusion of clipping of both common carotid arteries lasted for 10 min. Sham-control rats were produced by electrocoagulation of bilateral vertebral arteries followed by confirmation that both common carotid arteries were without ligation.

The carotid clamps were removed after 10-min clipping-4VO treatment [2,12]. The ACC perfusates were collected at 20-min intervals for 2 h during this experiment. All perfusates were analyzed for the quantitative release of DA and 5-HT in the ACC of rats treated with 4VO treatment, using high-performance liquid chromatography (HPLC)-electrochemical detection (ECD).

2.1.4. HPLC – ECD conditions

The collection of microdialysis perfusates was initiated approximately 2 h after inserting the probe and circulating an artificial cerebrospinal fluid (aCSF) solution (in mM: NaCl, 145; KCl, 2.7; $MgCl_2$, 1.0; $CaCl_2$, 1.2; containing 0.2 mM ascorbate and with the pH adjusted to 7.2–7.4 with 2 mM sodium phosphate buffer) through the probe at a flow rate of 0.8 μL/min. Perfusate samples were collected every 20 min into microcentrifuge tubes containing 5 μL of 0.1 N HCl and 5 μL of 0.1 M ethylenediaminetetraacetic acid (EDTA)-2Na and analyzed simultaneously. The aliquots (5 μL) were assayed for DA and 5-HT by a previously described procedure that was modified for the small-bore HPLC-ECD (EP-700, Eicom Corporation, Kyoto, Japan) [7].

The mobile phase consisted of 5% (v/v) acetonitrile, 13% (v/v) methanol, 0.1 M sodium dihydrogen phosphate, 0.2 mM 1-octanesulfonic acid sodium salt (SOS), and 0.12 mM EDTA-2Na (with pH adjusted to 2.8 with phosphoric acid). A C_{18} reversed-phase column (TSK-gel OSD-80Ts, 150 × 2.0 mm, 5-μm particle diameter, Tosoh Co., Tokyo, Japan) was used at a flow rate of 0.2 mL/min. The ECD was usually set at a sensitivity of 0.5 nA/v with a 0.6 V potential [13].

2.2. Results: experiment 1

The levels of DA and 5-HT releases in the ACC of sham-control rats were 430 (fM/20 min) and 190 (fM/20 min), respectively (Fig. 1). These quantitative values were used as the relative baseline levels for brain microdialysis analysis (Fig. 2).