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## HYPOXIA-INDUCED LONG-TERM POTENTIATION IN THE VESTIBULAR NUCLEUS

HIDEYUKI OKAMOTO, TOSHIAKI YAMANAKA and HIROSHI HOSOI
*Department of Otorhinolaryngology and Head & Neck Surgery, Nara medical University*
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*Abstract* : Transient ischemia due to a decrease in vertebrobasilar insufficiency (VBI) induces the hypoxia of vestibular nucleus (VN) and frequently cause vertigo. Furthermore, it is occasionally experienced that dizziness continues in the long term after strong rotatory vertigo in VBI. Since glutamate is thought to play an important role in the neurotransmission of VN, this study was undertaken to examine the effect of hypoxia on the VN neuron and the role of the glutamate in the hypoxia induced neuronal activities using electrophysiological and microiontophoretic technique. Cats anesthetized with α-chloralose were ventilated with a respirator. A silver recording microelectrode was inserted into the VN and the spontaneous firing of the neurons was continuously recorded on an ink-writing recorder through a spike counter. Micropipettes attached along the microelectrode were used for microiontophoretic application of 6,7-Dinitroquinoxaline-2,3-dione (DNQX), non-NMDA receptor antagonist and (+) 5-methyl-10,11-dihydro-5H-dibenzo (a,d) cyclohepten- 5,10-imine (MK801), NMDA receptor antagonist. The spontaneous firing in VN neurons increased transiently and then decreased, resulting in the disappearance in firings during 3 minutes inhalation of 5%$O_2$. However, the firings appeared again and persistently increased after the cessation of 5%$O_2$. Transient increase of the firing during hypoxia and persistent increase after the cessation of hypoxia were herein termed Hypoxic Depolarization (HD) and Post Hypoxic Potentiation (PHP), respectively. HD was significantly ($P<0.01$) suppressed by DNQX and MK801. Since HD was suppressed by glutamate receptor antagonist, HD was assumed to be caused by excessive glutamate released from presynaptic terminals in the VN neurons. HD correlated significantly with PHP ($R=0.609$, $p<0.01$). We indicate that enhancement of PHP was the change of glutamate receptor-mediated synaptic plasticity caused by HD in the VN neurons. In conclusion, it is suggested that HD and PHP shown in this electrophysiological study might imply possible mechanism underlying the onset of acute vertigo and persistent dizziness in VBI.

**Key words** : vestibular nucleus, hypoxia, glutamate, electrophysiological technique, long-term potentiation

## INTRODUCTION

Transient ischemia due to a decrease in blood flow of vertebral basilar artery (ex. vertebrobasilar insufficiency; VBI) induces the hypoxia of the central vestibular system and frequently cause vertigo and dizziness which occasionally last for a longer term in some cases even after the event.[1] For this reason why vertigo occur as an early symptom[2], vestibular nucleus (VN) neuron seems to have higher vulnerability to ischemia or hypoxia than the other areas of brain stem[3], since VN neuron, a key center of central vestibular system, is closely involved in the onset of vertigo and dizziness.[4] On the other hand, the CA1 hyppocampal neuron is known to be highly vulnerable to anoxia or ischemia. This vulnerability is suggested to relate with glutamate since anoxic episodes generate a selective delayed degeneration[5,6] or dysfunction[7] of pyramidal neurons which is inhibited by glutamate antagonists[8,9] Since glutamate is also thought to play an important role in the neurotransmission of VN,[10-15] VN is also likely to be vulnerable to hypoxia. However it is unclear whether and how VN neurons respond to hypoxic condition.

Therefore this study was undertaken to examine the effect of hypoxia on the VN neuron and the role of the glutamate in the hypoxia induced neuronal activities using *in vivo* electrophysiological and microiontophoretic technique.[16,3]

## MATERIAL AND METHODS

Adult healthy cats of both sexes weighing 2.5-4.9 kg were anesthetized with ketamine HCL (40 mg, i. m.). After cannulating the trachea and femoral vein, surgical procedures were carried out under $\alpha$-chloralose (30 mg/kg, i. v.) anesthesia. Each cat was ventilated with room air using a respirator (Narishige, Tokyo, Japan). All wound edges and pressure points were locally anesthetized with 1% lidocaine throughout the experiment. Supplemental doses of 10 mg/kg i. v. $\alpha$-chloralose were injected when as required. Under these anesthetized conditions, the animals did not appear to experience any pain or discomfort. Body temperature was maintained at 36.5-37.5 °C with a heating pad placed under the cat. Hypoxia was induced by ventilating the cat with a gas mixture of 5% O2 and 95% N2 for 3 minutes. The effects of hypoxia were examined only once in each cat. Blood pressure in the femoral artery was continuously recorded. PaO2, PaCO2, and pH were determined in arterial blood samples taken before and 2.5 minutes after the start of 5%O2 inhalation using an acid-base analyzer (ABL-30, Radiometer, Copenhagen, Denmark). After fixing the head of the animal in a stereotaxic instrument (SN-2, Narishige, Tokyo, Japan), the occipital skull and bony tentorium were removed to allow the insertion of recording electrode. The stereotaxic apparatus was placed on a turntable that could be manually rotated sinusoidally in a horizontal plane for 120° at an angular speed of approximately 60°/s. A glass-insulated silver wire microelectrode (electrical resistance, approximately 1 MΩ; outer diameter, approximately 10 $\mu$m) was inserted into the VN (P, 8.5; L, 2.5, H, 2.5-4.5 mm from cortical surface according to the brain map of Snider and Niemer17) ). A seven-barreled micropipette attached along the recording microelectrode was used for microiontophoretic application of drugs. Each barrel was filled with 1M monosodium

L-glutamate (Sigma), 20 mM 6,7-Dinitroquinoxaline-2,3-dione (DNQX) (Sigma), non-N-methyl-D-aspartate (NMDA) receptor antagonist, and 50 mM (+) 5-methyl-10,11-dihydro-5H-dibenzo (a,d) cyclohepten- 5,10-imine (MK801) (Sigma), NMDA receptor antagonist. These drugs were iontophoretically applied to the immediate vicinity of the target neurons being recorded using an iontophoresis programmer (SEZ-1100; Nihon Kohden, Tokyo, Japan) constant microcurrent supply. Schematic experimental procedure was summarized in Fig. 1. The spontaneous firing of the neurons was continuously recorded on an ink-writing recorder (RJG-4022, Nihon Kohden, Tokyo, Japan) through a spike counter (DSE-325P, Dia Medical System, Tokyo, Japan). DNQX and MK801 at dose of 20 nA, respectively were administered between 30 seconds before the start of 5%O2 inhalation and the end of the inhalation. After the termination of each experiment, the position of the recording electrode was marked by passing an anodal current of 0.1 mA for 1 minute and histologically checked using cresyl violet stain. Statistical significance was determined using Student's t-test. Further details of the experimental procedures have been reported elsewhere.[6,17] This study was approved by the Animal Research Committee of Nara Medical University.

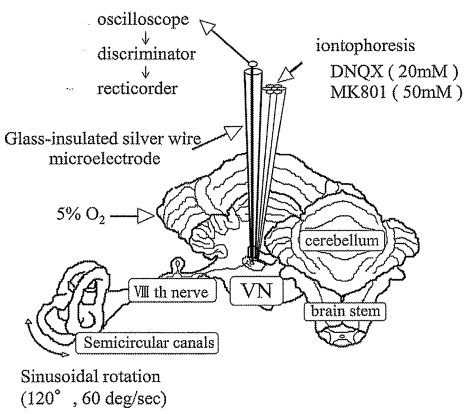


Fig. 1 : Schema of this experiment
Each cat was ventilated with room air using a respirator. Hypoxia was induced by ventilating the cat with a gas mixture of 5% $O_2$ and 95% $N_2$ for 3 minutes. Each cat was placed on a turntable that could be manually rotated sinusoidally in a horizontal plane for 120° at an angular speed of approximately 60°/s. A glass-insulated silver wire microelectrode (electrical resistance, approximately 1 MΩ; outer diameter, approximately 10 $\mu$m) was inserted into the VN. A seven-barreled micropipette attached along the recording microelectrode was used for microiontophoretic application of drugs. Each barrel was filled with 1M monosodium L-glutamate, 20 mM DNQX and 50 mM MK801. These drugs were iontophoretically applied to the immediate vicinity of the target neurons being recorded using an iontophoresis programmer.
Abbreviation : VN, vestibular nucleus; DNQX, 6,7-Dinitroquinoxaline-2,3-dione , non-N-methyl-D-aspartate (NMDA) receptor antagonist; MK801, (+) 5-methyl-10,11-dihydro-5H-dibenzo (a,d) cyclohepten- 5,10-imine, NMDA receptor antagonist

## RESULTS

Eighty two neurons which were located histologically in the VN were classified into Type I, II, III and IV, according to their responses to horizontal rotation. Type I neurons exhibited briefly an increase and a decrease in firings in response to horizontal rotations ipsilateral and contralateral to the recording site, respectively. In contrast to type I, type II neurons showed opposite responses. Furthermore, type III and type IV neurons displayed an increase and a decrease in neuronal activities in response to bilateral horizontal rotation. Based on this classification, 65 and 17 neurons of the 82 VN neurons were identified as type I and II, respectively. Type III and IV neurons were not obtained in any of the 82 VN neurons recorded. The effects of hypoxia were examined on type I neurons (n = 65) which primarily receive inputs from the vestibular nerve and project fibers to occulomotor nuclei in vestibulo-ocular reflex. $PaO_2$ and $PaCO_2$ were analyzed just before and 3 minutes after the start of 5% $O_2$ inhalation in every cat, and the changes of $PaO_2$ were not significant. Whereas, the mean

$PaCO_2$ were slightly increased by hypoxia, respectively, and the changes of $PaCO_2$ were not significant (Table 1). The mean $PaO_2$ values in all cats decreased by hypoxia from 120.6 ± 2.6 to 33.7 ± 1.1. Among control (n = 39), DNQX applied cats (n = 13) and MK801 applied cats (n = 13) there were no significant differences in $PaO_2$ and $PaCO_2$ values before the start or at the end of hypoxia as shown in Table 1.

| | $PaO_2$ (mm Hg) | | $PaCO_2$ (mm Hg) | |
|---|---|---|---|---|
| | before hypoxia | hypoxia | before hypoxia | hypoxia |
| Control | 121.2±3.0 | 34.7±1.3 | 36.2±1.7 | 38.6±1.2 |
| DNQX | 117.7±4.16 | 30.3±2.7 | 33.4±2.1 | 36.3±2.6 |
| MK801 | 122.2±7.1 | 32.9±2.9 | 33.8±2.8 | 37.5±2.4 |
| Total | 120.6±2.6 | 33.7±1.1 | 35.1±1.2 | 37.9±1.0 |

Table 1 : Effects of inhalation of 5% $O_2$ on Arterial Blood Gases in Cats (mean ± SE)
The mean $PaO_2$ in all cats, control, application of DNQX and application of MK801 were decreased by hypoxia, respectively, and the changes of $PaO_2$ were not significant. Whereas, the mean $PaCO_2$ were slightly increased by hypoxia, respectively, and the changes of $PaCO_2$ were not significant

The spontaneous firing in VN type I neurons increased transiently, then gradually decreased and disappeared during 3 minutes inhalation of 5% $O_2$. The spontaneous firing appeared again and persistently increased after the cessation of 5% $O_2$ (Fig. 2). Transient increase of the spontaneous firing during hypoxia was termed Hypoxic Depolarization (HD). The rate of number of spikes on HD peak to spikes on the baseline before hypoxia was expressed as the HD index. Persistent increase of the spontaneous firing after the cessation of hypoxia was referred to as Post Hypoxic Potentiation (PHP). The rate of number of spikes 30 minutes after the start of 5% $O_2$ inhalation to spikes on the baseline before hypoxia was expressed as the PHP index. The relationship between HD index and disappearance time of firing after the hypoxia was investigated by Pearson correlation analysis (Fig. 3). HD index correlated significantly with disappearance time of firing after the hypoxia ($R = 0.586$, $p = 0.00043$). The mean HD index by application of DNQX and MK801 was significantly ($P < 0.01$) suppressed from 2.28 ± 0.18 to 1.49 ± 0.18 and 1.48 ± 0.21, respectively (Fig. 4). The mean disappearance time of firing by application of DNQX and MK801 was significantly ($P < 0.01$) reduced from 2.56 ± 0.28 minute to 1.46 ± 0.26 minute and 1.55 ± 0.19 minute, respectively (Fig. 5). The relationship between HD index and PHP was investigated by Pearson correlation analysis (Fig. 6). HD index correlated significantly with PHP index ($R = 0.609$, $p = 0.0032$). The mean PHP index by application of DNQX and MK801 was significantly ($P < 0.01$) inhibited from 1.24 ± 0.10 to 0.94 ± 0.09 and 0.92 ± 0.10, respectively (Fig. 7).

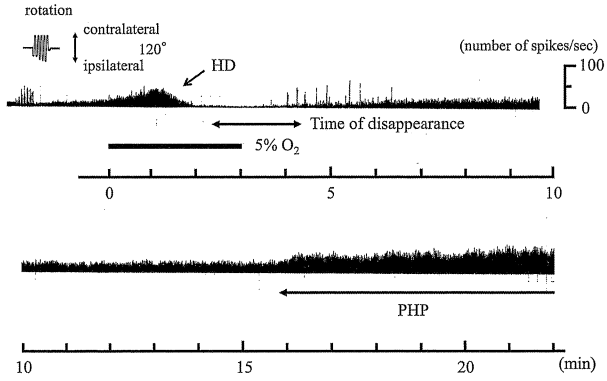


Fig. 2 : Example of the effect of inhalation of 5% $O_2$ for 3 minutes on spontaneous firing of a neuron in vestibular nucleus.
The spontaneous firing in VN neurons increased transiently, then gradually decreased and disappeared during 3 minutes inhalation of 5% $O_2$. The spontaneous firing appeared again and persistently increased after the cessation of 5% $O_2$. Transient increase of the spontaneous firing during hypoxia was termed Hypoxic Depolarization (HD). Persistent increase of the spontaneous firing after the cessation of hypoxia was referred to as Post Hypoxic Potentiation (PHP).
Abbreviation : Hypoxic Depolarization , HD; PHP, Post Hypoxic Potentiation

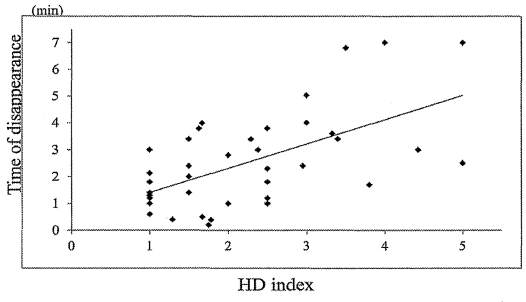


Fig. 3 : Regression analyses of time of disappearance and HD index. A positive correlations of time of disappearance and HD index was seen (R=0.586, P=0.00043, n=39). The rate of number of spikes on HD peak to spikes on the baseline before hypoxia was expressed as the HD index.

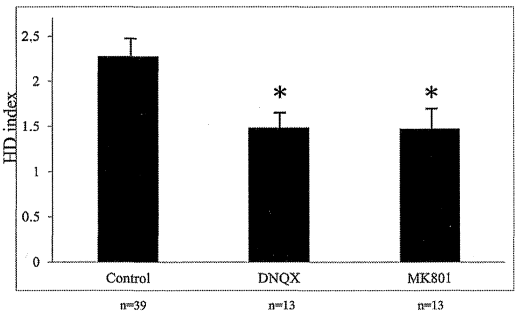


Fig. 4 : Effects of DNQX and MK801 for HD index. Each value represents the mean ± SE. Asterisks indicate significant differences from the control level (P < 0. 01, Student's *t* –test).

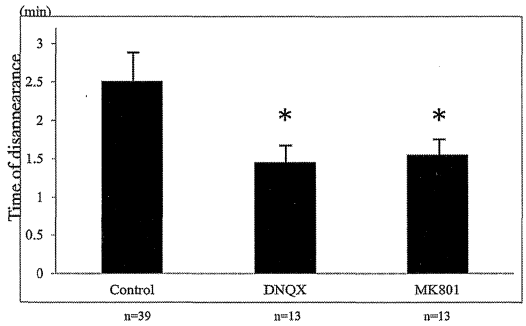


Fig. 5 : Effects of DNQX and MK801 for Time of disappearance. Each value represents the mean ± SE. Asterisks indicate significant differences from the control level (P < 0. 01, Student's *t* –test).

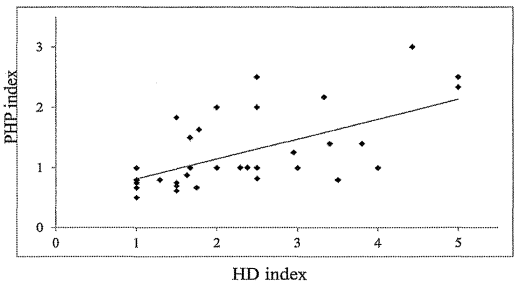


Fig. 6 : Regression analyses of PHP index and HD index. A positive correlations of PHP index and HD index was seen (R=0.609, P=0.0032, n=39 ). The rate of number of spikes 30 minutes after the start of 5% $O_2$ inhalation to spikes the baseline before hypoxia was expressed as the PHP index.

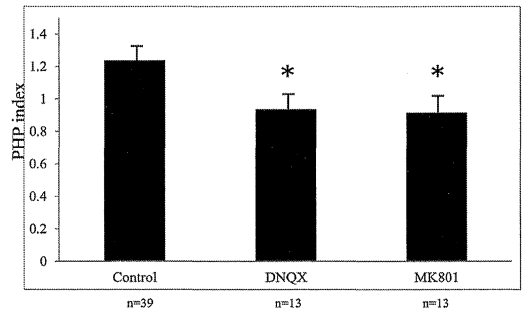


Fig. 7 : Effects of DNQX and MK801 for PHP index. Each value represents the mean ± SE. Asterisks indicate significant differences from the control level (P < 0. 01, Student's *t* –test).

## DISCUSSION

Vertigo and dizziness is well known to be the most common symptom caused by VBI.[1,18] VN is a key area in the vestibular nervous system closely connected with the incidence of vertigo and dizziness.[4] Since previous histological[19] and electrophysiological[3] studies showed that VN neurons were vulnerable to ischemia and hypoxia, vertigo seems likely to occur on VBI. Furthermore, it is occasionally experienced that dizziness continues for the long term after strong rotatory vertigo under VBI.[1] This electrophysiological study was therefore undertaken to elucidate the mechanism of VN neuronal activity due to hypoxia *in vivo* experiment. In the present study, the firing in VN neurons showed HD, followed by a disappearance due to hypoxia. Since the disappearance time of firing in VN neuron induced by hypoxia was longer as HD index was higher, it is suggested that the functional impairment of VN neuron is affected by the intensity of HD. Because HD was suppressed by glutamate receptor antagonist, HD was assumed to be caused by excessive glutamate released from presynaptic terminals in the VN neurons.

It has been suggested that glutamate is involved in VN neuronal damage, as glutamate is the primary afferent neurotransmitter from the vestibular nerve to the VN neuron.[10,11,14,16] We indicate that enhancement of PHP was the change of glutamate receptor-mediated synaptic plasticity caused by HD in the VN neurons. As for long-term potentiation (LTP) caused by the tetanic stimulation in the CA1 hyppocampal neuron,[20] $Ca^{2+}$ dependent protein kinase such as CaMkII,[21,22] PKA,[23] and protein kinase C(PKC),[24-26] and protein phosphatase such as calcineurin are activated by explosive $Ca^{2+}$ influx in the postsynaptic cell[27] mediated by glutamate receptor,[14] as a result, various transmission mechanism about LTP is induced. There are the reports of LTP induced by anoxia regarded as the same mechanism of LTP induced by the tetanic stimulation in the CA1 hyppocampal neuron.[8,24,28-31] This form of LTP was referred as anoxic LTP which is mediated by a persistent upregulation of postsynaptic NMDA receptors-mediated currents. It is hypothesized that hypoxic LTP phenomenon might occur in VN neuron similar to the CA1 hyppocampal neuron.[8,24,28-31] This hypothesis could supports our results in the current study suggesting that the PHR relates to glutamate receptors in the VN.

In conclusion, although further research is required to confirm these preliminary results obtained from the present *in vivo* study, HD and PHP shown in this electrophysiological study might suggest posssible mechanism underlying the onset of acute vertigo and persistent dizziness in VBI.

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