Different Sensitivity to Hypoxia in Neuronal Activities of Lateral Vestibular and Spinal Trigeminal Nuclei

Shinzo Yoshida, MD, Masashi Sasa, MD, and Shuji Takaori, MD

Electrophysiologic studies were performed to examine the effects of hypoxia on neuronal activities of the lateral vestibular and spinal trigeminal nuclei using rats anesthetized with chloral hydrate. The rats inhaled a gas mixture of 5% oxygen and 95% nitrogen for 3.5 minutes to induce hypoxia, followed by room air. Under these conditions, mean Pao2 was decreased from 85 to 22 mm Hg 3 minutes after the start of the inhalation concomitant with a decrease in blood pressure from 108 to 55 mm Hg. There were no significant differences in these variables between rats used for vestibular nucleus experiments and rats used for trigeminal nucleus experiments. In the lateral vestibular nucleus, hypoxia inhibited postsynaptic components of the evoked field potential, spike generation of monosynaptic neurons on vestibular nerve stimulation, and firing induced by iontophoretic application of glutamate. In the spinal trigeminal nucleus, however, there were no alterations of the field potential or spike generation of the neurons on trigeminal nerve stimulation. These results indicate that the lateral vestibular nucleus neurons are much more sensitive to hypoxia than the spinal trigeminal nucleus neurons. The failure of transmission in the monosynaptic neurons of the lateral vestibular nucleus is suggested to be due to the inhibition of excitability of the postsynaptic membrane. (Stroke 1988;19:357-364)

Vertigo is known to occur during transient ischemic attacks due to a decrease in blood flow through the blood vessels supplying the vestibular nuclear regions such as the anterior and posterior inferior cerebellar arteries. Vertigo and dizziness are also early symptoms of cerebral hypoxia. In experimental animals, the drugs effective in treatment of vestibular disorders, such as diphenhydramine, beta-histidine, and ciperione, inhibited neuronal activities in the vestibular nuclei. In contrast, cinnarizine and ATP, which are also effective anti-vertigo drugs and increase cerebral blood flow, enhanced neuronal activities of the vestibular nuclei. Since vertigo is caused by an imbalance between the vestibular function of right and left sides, vertigo resulting from unilateral hyperfunction or hypofunction is considered to be effectively treated with the drugs that respectively inhibit or enhance the lateral vestibular nucleus (LVN) neuron activities. Ethanol, which is known to induce equilibrium disorders concomitant with nystagmus, inhibits synaptic transmission of LVN neurons monosynaptically activated by vestibular nerve stimulation with little effect on the spinal trigeminal nucleus (STN) neurons or on the principal cells of the lateral geniculate nucleus. There are several reports supporting the hypothesis that acetylcholine functions as a neurotransmitter in the relay from the vestibular nerve to the LVN mono-synaptic neurons and that the neurologic impairment during a hypoxic state is due to suppression of the cholinergic system.

These results suggest that the neurons in the vestibular nuclei are more sensitive to cerebral ischemia and hypoxia than other nuclei in the brainstem and that clinical symptoms such as vertigo and equilibrium disorders might be explained by the functional vulnerability of these neurons under such conditions. We electrophysiologically examined the effects of hypoxia on the LVN and STN neurons, which are primary sensory nuclei, since ischemia in the vertebrobasilar arterial system induces clinical symptoms in both nuclei.

Materials and Methods

Male Wistar rats weighing 310–350 g were anesthetized with 300 mg/kg i.p. chloral hydrate. After tracheal cannulation, each rat was immobilized with 100 mg/kg i.p. gallamine triethiodide and artificially ventilated with room air using a respirator (AR-2, Narishige, Tokyo, Japan). All wound edges and pressure points were locally anesthetized with 8% lidocaine spray. Supplemental doses of 150 mg/kg i.p. chloral hydrate were injected when the experiment exceeded 4 hours. Body temperature was maintained at 36.5–37.5°C with a heating pad placed under the rat.

Hypoxia was induced by ventilating the rat with a gas mixture of 5% O2 and 95% N2 for 3.5 minutes, followed by room air. The effects of hypoxia were examined only once in each rat. The field potentials and spikes of single neurons elicited by vestibular nerve stimulation were recorded before, during, and after the inhalation of 5% O2. Blood pressure in the femoral artery was continuously recorded. PaO2, PaCO2, and pH were determined in arterial blood samples taken before and 3 minutes after the start of 5% O2 inhalation using an

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TABLE 1. Distribution of Neurovascular Risk Factors in 40 Patients With Cerebral Ischemic Events

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>MVP (n = 24)</th>
<th>No MVP (n = 16)</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Nicotine abuse</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>No risk factor</td>
<td>20</td>
<td>4</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

*MVP, mitral valve prolapse.*

All subjects included gave their informed consent, and the study was performed respecting the principles outlined in the declaration of Helsinki.

**Echocardiographic Technique**

The echocardiographic examination was performed using a Diasonic 6400 phased-array sector scanner (Palo Alto, California). Within 3–4 weeks after the cerebral ischemic event, the patients were submitted to conventional TTE performed by two echocardiographic examiners with no knowledge of the patient’s history. TTE examinations used the parasternal and apical acoustic windows. The diagnosis of MVP was made using the criteria proposed by Markiewicz et al11 in the one-dimensional echocardiogram and by Mor- ganroth et al12 in the two-dimensional echocardiogram. The echocardiograms were recorded with a Panasonic video recorder and analyzed frame by frame using two independent observers who were not familiar with the patient’s history. Valves were considered abnormal if the prolapsing mitral valve appeared thickened, shaggy, and redundant on more than one examination plane and club-shaped in the long-axis parasternal view triggered in diastole.

**Transesophageal echocardiography.** All patients and controls underwent a TEE examination using an echoscope developed by Hanrath et al13 in which a special 3.5-MHz transducer array of 32 elements is fitted to the distal end of a gastroscope with an outer diameter of 9 mm. Patients fasted for approximately 16 hours before undergoing TEE. Local anesthetic was applied to the pharyngeal region before examination. Since a 3.5-MHz transducer had been fixed in place of the fiber optic system of a conventional gastroscope, patients suspected of having esophageal disease swallowed barium under x-ray control to exclude a diverticulum of the esophagus. Three patients were excluded from this study because they were not able to swallow the echoscope. The examination lasted between 20 and 25 minutes, with no side effects. As with TTE, the whole examination was recorded with a video recorder and analyzed by two independent observers for semiquantitative statements.

The transesophageal measurements of the mitral valve were performed in a standardized transesophageal examination plane at 10 cm scanning depth (Figure 1) and were taken by one independent observer who was blinded to the origin of the videotapes. The prolapse of the mitral leaflets was measured from a line between the leaflet coaptation point and the mitral annulus to the point of maximum prolapse toward the left atrium. MVP was assumed if one or both mitral leaflets prolapsed at a minimum of 3 mm over the valve plane into the left atrium. The leaflet thickness was measured in late systole, triggered by electrocardiography at the end of the T

![Figure 1](http://stroke.ahajournals.org/).
FIGURE 2. Effects of inhalation of 5% O2 for 3.5 minutes on mean arterial blood pressure in 17 and 12 rats used for experiments on lateral vestibular nucleus (●) and spinal trigeminal nucleus (○), respectively. Each point indicates mean; each vertical bar SEM. **p<0.01 significantly different from before inhalation.

The field potential in the LVN evoked by vestibular nerve stimulation consisted of three negative components, P, N1, and N2 waves, as demonstrated in Figure 3, top. The mean latencies of the peaks of P, N1, and N2 waves in six rats were 0.31, 1.04, and 2.05 msec, respectively; the mean amplitudes of the three waves were 272, 385, and 90 μV, respectively. Hypoxia produced a gradual and significant decrease in the amplitude of the N1 and N2 waves without affecting the amplitude of the P wave. During the recovery period, the amplitude of the N1 wave returned to the control level within 1 minute, but that of the N2 wave did not return in 5 minutes. The latencies of the N1 and N2 waves were slightly prolonged during hypoxia, but the changes in latencies were not significant.

The field potential in the STN elicited by tooth pulp stimulation consisted of two negative components, N1 and N2 waves, corresponding to the presynaptic and postsynaptic components, respectively, as previously reported. The mean latencies of the peaks of the N1 and N2 waves in five rats were 0.53 and 1.21 msec, respectively; the mean amplitudes of the two waves were 266 and 74 μV, respectively. The latencies and amplitudes of the two waves were unaffected by hypoxia, as shown in Figure 3, bottom.

Monosynaptic and polysynaptic neurons in the LVN were classified by stimulating the vestibular nerve in rats as done previously in cats. When stimulus was applied to the nerve, the latency of evoked spikes for the monosynaptic neuron was approximately 1.0 msec, while that for the polysynaptic neuron was > 2.0 msec. In our experiments, the effects of hypoxia were tested on the monosynaptic neurons.

### Table 1. Effects of Inhalation of 5% O2 on Arterial Blood Gases in Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Pao2 (mm Hg)</th>
<th>Paco2 (mm Hg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Hypoxia</td>
<td>Control</td>
</tr>
<tr>
<td>Lateral vestibular nucleus</td>
<td>11</td>
<td>86 ± 3</td>
<td>23 ± 1</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>Spinal trigeminal nucleus</td>
<td>12</td>
<td>85 ± 2</td>
<td>22 ± 1</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>85 ± 2</td>
<td>22 ± 1</td>
<td>32 ± 2</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n, number of rats. Control and hypoxia show the results before and 3 minutes after inhalation of 5% O2, respectively.
Spike generation of the monosynaptic neurons on vestibular nerve stimulation was inhibited by hypoxia within 1.5 minutes but returned to normal during the recovery period after 10 minutes (Figure 4, left). A significant ($p < 0.01$) inhibition of the number of spikes was observed in eight of 12 LVN neurons during hypoxia. The mean ± SEM number of spikes in the 12 neurons was significantly reduced, from $1.47 ± 0.26$ to $0.54 ± 0.14$ 3 minutes after the inhalation of 5% O₂ (Table 2).

In contrast to the LVN neurons, hypoxia had no effect on the spike generation elicited by tooth pulp stimulation in any of seven STN neurons tested (Figure 4, right).

The effects of hypoxia on spontaneous firing were examined in 17 LVN neurons, which were monosynaptically activated by vestibular nerve stimulation. In 12 neurons, spontaneous firing was transiently increased 10–40 seconds after the start of inhalation, then gradually reduced, and finally disappeared in 2–3 minutes (Figure 5, top). A decrease in firing rate without a transient increase was seen in the remaining five LVN neurons (Figure 5, middle). In contrast, a transient increase in spontaneous firing was seen 10–40 seconds after the start of inhalation in only one of 10 STN neurons examined. In the remaining nine neurons, spontaneous firing was gradually reduced without a transient increase during hypoxia (Figure 5, bottom). However, tooth pulp stimulation still generated spikes in the STN neurons in which spontaneous firing had been reduced by hypoxia.

The effects of hypoxia on glutamate-induced firing were also tested in 17 LVN neurons. The ejection current of glutamate in each neuron was in the range of 20–50 nA so as to obtain consistent firing rates of 50–70/sec during iontophoretic application for 5 sec-
TABLE 2. Effects of Inhalation of 5% O₂ on Number and Latency of Spikes of Lateral Vestibular Nucleus Neurons and Spinal Trigeminal Nucleus Neurons

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1.5 min</th>
<th>3 min</th>
<th>10 min after cessation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lateral vestibular nucleus (n = 12)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of spikes</td>
<td>1.47 ± 0.26</td>
<td>0.68 ± 0.16*</td>
<td>0.54 ± 0.14*</td>
<td>1.69 ± 0.47</td>
</tr>
<tr>
<td>Latency (msec)</td>
<td>1.14 ± 0.08</td>
<td>1.46 ± 0.27</td>
<td>1.89 ± 0.43</td>
<td>1.20 ± 0.13</td>
</tr>
<tr>
<td><strong>Spinal trigeminal nucleus (n = 7)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of spikes</td>
<td>1.79 ± 0.51</td>
<td>1.86 ± 0.54</td>
<td>2.31 ± 0.67</td>
<td>1.51 ± 0.34</td>
</tr>
<tr>
<td>Latency (msec)</td>
<td>2.85 ± 0.75</td>
<td>2.11 ± 0.56</td>
<td>3.18 ± 1.14</td>
<td>3.07 ± 0.82</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n, number of neurons.
*pSignificantly different from control, p < 0.05.

onds. The increase in firing induced by glutamate was suppressed by hypoxia in 13 neurons (Figure 5, top and middle). In the remaining four neurons, the reduction of glutamate-induced firing during hypoxia was <50% of the control level. Figure 6 shows the time course of the effects of hypoxia on the mean glutamate-induced firing rate in 17 neurons. A significant (p < 0.01) decrease in firing rate was observed 2 minutes after the start of inhalation of 5% O₂, and continued for 5 minutes after the cessation of inhalation. Partial recovery of the firing rate was noted 10 minutes after the cessation of inhalation.

Iontophoretically applied acetylcholine at doses of 50–100 nA increased the firing rate in five of 17 LVN neurons, but the remaining 12 neurons were not affected by the drug (Figure 5, top and middle). An inhibition of the glutamate-induced firing during hypoxia was observed in three of the five cholinoceptive neurons and in 10 of the 12 noncholinoceptive neurons. There were no apparent differences of sensitivity to hypoxia between the cholinoceptive and noncholinoceptive neurons.

**Discussion**

When 5% O₂ was inhaled by rats for 3.5 minutes, Pao₂ was reduced to 20–30% of the control value simultaneously with a lowering of the arterial blood pressure. Energy metabolism is reported to be negligibly affected by such a lowering of Pao₂ alone. However, the possibility cannot be excluded that the concomitant

FIGURE 4. Effects of inhalation of 5% O₂ for 3.5 minutes on spike generation of monosynaptic neuron in lateral vestibular nucleus (left) on vestibular nerve stimulation and spinal trigeminal nucleus (right) on tooth pulp stimulation. ▲ indicates stimulus artifact.
hypotension seen in our hypoxia model further aggravated energy metabolism already at a critical level and that there was impairment in cerebral energy status. In contrast to $\text{Pao}_2$, $\text{Paco}_2$ was reduced to only 78% of the control level and pH was unaltered during hypoxia. This appears to be due to the short duration of inhalation of 5% $\text{O}_2$.

**Figure 5.** Effects of inhalation of 5% $\text{O}_2$ for 3.5 minutes on spontaneous and glutamate-induced firing of 2 monosynaptic neurons in lateral vestibular nucleus (top and middle) and spinal trigeminal nucleus neuron (bottom). Acetylcholine ($\text{ACh}$, 50 nA) and glutamate ($\text{G}$, 40 nA) were iontophoretically applied during periods indicated by broken and solid lines, respectively.

**Figure 6.** Time course of effects of inhalation of 5% $\text{O}_2$ for 3.5 minutes on glutamate-induced firing of 17 monosynaptic neurons in lateral vestibular nucleus. Each point indicates mean number of spikes/sec and vertical bar SEM. *$p<0.05$, **$p<0.01$ significantly different from value before inhalation.
Under this condition, we observed an inhibition of both monosynaptic (N, wave) and polysynaptic (N, wave) components of the field potential as well as inhibition of spike generation of the monosynaptic neurons in the LVN on vestibular nerve stimulation. In contrast, the field potential and spike generation in the STN on tooth pulp stimulation was unaffected by hypoxia. No difference in $P_aO_2$, $P_aCO_2$, or pH was found between rats used for experiments on the LVN and STN. During hypoxia, the initial elevation followed by sustained lowering of the blood pressure was similarly observed in both LVN and STN groups. Since the different responses to hypoxia between LVN and STN neurons are probably not related to systemic factors such as arterial blood gas and blood pressure, it appears that the transmission failure seen in LVN neurons on vestibular nerve stimulation is due to the intrinsic character of the LVN neurons themselves.

However, it is also possible that reduction of blood flow during the hypotensive phase of hypoxia was more severe in the LVN area than in the STN area since we did not measure regional cerebral blood flow. This possibility, however, is unlikely for the following reasons. In humans, the LVN and STN areas are both supplied through the lateral branches of the vertebral artery, basilar artery, anterior inferior cerebellar artery, and posterior inferior cerebellar artery and are regarded as belonging to the same arterial territory. In addition, the density of the capillary network in these nuclei is reported to be similar in humans, although there has been no detailed study in rats.

Lastly, we have previously demonstrated that cinnarizine, which increased cerebral blood flow while lowering blood pressure, enhanced the neuronal activities of the LVN neurons without affecting those of the STN neurons. Therefore, STN neurons are considered to be much less sensitive to changes of blood flow, and hence hypoxia, than LVN neurons.

The inhibition of spike generation in the LVN neurons on vestibular nerve stimulation during hypoxia is considered to result from one or more of the following reasons: first, conduction block of the afferent vestibular nerve may have occurred; second, excitability of the postsynaptic membrane may have been depressed; third, release of neurotransmitter from the presynaptic terminals may have been inhibited; fourth, receptors for neurotransmitters on the postsynaptic membrane may have been impaired; and finally, synthesis of neurotransmitter in the presynaptic terminals may have been inhibited. The first possibility can be excluded since axons are known to be relatively insensitive to anoxia or hypoxia. In addition, our results, that the presynaptic component (P wave) of the field potential in the LVN was unaffected by hypoxia, also indicate that conduction block of the vestibular nerve does not occur.

The second possibility is the most probable for the following reasons. During hypoxia, the time course of the inhibitory effect on firing induced by glutamate was similar to that of the inhibition of spikes elicited by vestibular nerve stimulation. Since glutamate is known to depolarize the postsynaptic membrane, the inhibition of transmission in the LVN monosynaptic neurons during hypoxia is suggested to be due to depressed excitability in the postsynaptic membrane. Depression of LVN neurons may be explained by the mechanism proposed by Hansen et al using the hippocampal slice preparation, that anoxia produced hyperpolarization primarily due to an increase in $K^+$ conductance. However, the third possibility, that the presynaptic terminals were simultaneously inhibited, should be taken into consideration since depolarizing block of the presynaptic terminals in the spinal motoneuron as well as an inhibition of release of acetylcholine has been reported under moderate hypoxia similar to ours and asphyxia.

We also found an inhibition of glutamate-induced firing of both cholinoceptive and noncholinoceptive LVN neurons during the inhalation of 5% $O_2$. The fourth possibility, that hypoxia selectively affects the cholinoceptive receptors in the postsynaptic membrane of LVN neurons (in which acetylcholine appears to be involved in the primary afferent transmission), is, therefore, unlikely. However, the fifth possibility cannot be completely excluded because the impairment of acetylcholine synthesis has also been reported.

During hypoxia, spontaneous firing in the LVN neurons transiently (10–30 seconds) increased, then gradually decreased, and finally disappeared. The initial enhancement of neuron activities may be due to an increase in transmitter released from the presynaptic nerve terminals rather than postsynaptic hyperexcitability as suggested by Eccles et al since no increase in amplitude of the field potential or spikes elicited by vestibular nerve stimulation was observed at an early stage of hypoxia. Presumably, a transient increase in transmitter release is induced by a transient elevation of the extracellular $K^+$ concentration and subsequent depolarization of the nerve terminals, both of which have been observed soon after the initiation of anoxia. Hansen has reported that, following cardiac arrest, the extracellular $K^+$ concentration transiently (within 30 seconds) increases, then returns to the previous level, and thereafter starts to increase gradually in 1 minute. This change in the $K^+$ level appears to be parallel to that of spontaneous firing in LVN neurons during the early hypoxic period in our study.

In contrast to the LVN neurons, a transient increase in spontaneous firing of the STN neurons was rarely seen during hypoxia, although the firing gradually decreased. It is likely that an increase in the extracellular $K^+$ level is not sufficient to induce transmitter release from the presynaptic terminals of the STN neurons, as reported by Hansen. A decrease in spontaneous firing was also observed in the STN neurons 3–3.5 minutes after the start of hypoxia; however, spikes were still elicited by tooth pulp stimulation. Therefore, synaptic transmission and excitability of the STN neurons are considered to be not impaired under our hypoxic conditions. Bureš and Burešová reported no significant differences in the
elevation of the extracellular $K^+$ level among different brain sites within 3.5 minutes after respiratory arrest, although differences were seen after 3.5 minutes of asphyxia. Thus, it is unlikely that there are differences in the extracellular $K^+$ level between the STN and LVN areas in 3-3.5 minutes. Therefore, inhibition of the excitability of LVN neurons does not appear to be due to an increase in the $K^+$ level in the extracellular space but to intrinsic factors such as an increase in $K^+$ conductance and resultant hyperpolarization as mentioned above.

In conclusion, the LVN monosynaptic neurons are sensitive to hypoxia but the STN neurons are not.

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KEY WORDS • anoxia • rats • trigeminal nucleus, spinal • vestibular nucleus
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