EFFECTS OF DIPHENHYDRAMINE IONTOPHORETICALLY APPLIED ONTO NEURONS IN THE MEDIAL AND LATERAL VESTIBULAR NUCLEI

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Abstract—Electrophysiological studies were carried out to elucidate the effects of diphenhydramine, an antihistamine (H1-receptor blocking) drug, on neuron activities in the medial vestibular nucleus (MVN) and lateral vestibular nucleus (LVN) of cats using a microiontophoretic method. According to the firing pattern and latency of the first spike with vestibular nerve stimulation, neurons in the MVN and LVN were classified into two groups: monosynaptic and polysynaptic neurons. In the MVN, the spike generation of polysynaptic neurons was dose-dependently inhibited with the iontophoretic application of diphenhydramine up to 200 nA, and that of the monosynaptic neurons was also suppressed by the maximum dose of 200 nA. In contrast to the MVN neurons, the spike generation of LVN monosynaptic neurons remained unaffected with diphenhydramine up to 200 nA, although an inhibition of the LVN polysynaptic neurons was obtained with 200 nA of the drug. These results suggest that small doses of diphenhydramine more selectively interfere with synaptic transmission in the MVN neuron than that in the LVN neuron.

It has been generally accepted that motion sickness is produced by an imbalance in the central vestibular function or massive impulses from the peripheral organs. Diphenhydramine and dimenhydrinate, histamine H1-receptor blocking agents, are known to belong to a group of the most effective drugs that prevent motion sickness; however, the mechanism involved in the antimotion sickness action of these drugs remains to be determined. The effects of intravenous administration of these drugs on neuron activities of the vestibular nuclei have been reported by two groups: Jaju and Wang (1) reported that both diphenhydramine (1.5 mg/kg, i.v.) and dimenhydrinate (2.5–5.0 mg/kg, i.v.) suppressed the spontaneous as well as the enhanced neuronal firing; however, Sekitani et al. (2) found no effect of dimenhydrinate (2 mg/kg, i.v.) on the spontaneous neuronal activity of the medial vestibular nucleus (MVN). Thus, the effects of diphenhydramine on neuron activities in the MVN and lateral vestibular nucleus (LVN) were studied using a microiontophoretic method to determine the possible site of action of this drug.

Materials and Methods

Thirty-one adult cats of both sexes weighing 2.5–4.0 kg were used. All surgical procedures were performed with the animal under ether inhalation anesthesia. After cannulating the trachea and femoral vein, the left tympanic bulla was trepanned under a ventral approach. For vestibular nerve stimulation, a bipolar stainless steel electrode
with a tip diameter of 0.5 mm was inserted through the exposed round window and fixed with dental cement. The head of the animal was fixed in a stereotaxic instrument, and the occipital skull and bony tentorium were removed to allow insertion of a recording electrode. After the operation, the administration of ether was replaced by α-chloralose (30 mg/kg, i.v.) and immobilization with gallamine triethiodide (5 mg/kg/hr, i.v.). All wound edges and pressure points were locally anesthetized repeatedly with 8% lidocaine solution as required. Respiration was artificially sustained, and body temperature was kept at 36.5–37.5°C with a heating pad.

Stimuli composed of square wave pulses of 0.05 msec duration and 1–10 V intensity were applied to the vestibular nerve every 1.6 sec; intensity of the stimulus was 1.5 times higher than the threshold voltage. A glass-insulated silver wire microelectrode (electrical resistance: approx. 1 MΩ) attached along a seven-barreled micropipette was inserted to the left MVN (P: 8.0, L: 2.5, H: −2.0 to −3.0) and LVN (P: 8.0, L: 4.0, H: −3.5 to −4.5), according to the brain atlas of Snider and Niemer (3). The outer diameter of the micropipette was approximately 10 μm, and the distance between the tips of the recording electrode and micropipette was within 15 μm. The pipette was filled with 0.2 M diphenhydramine HCl (Kowa Co.), 2 M monosodium glutamate (Sigma) and 3 M NaCl; these chemicals were ejected to the immediate vicinity of the target neuron using a microiontophoresis programmer (WP-1, model 160). The mean spike number and latency of the first spike elicited by vestibular nerve stimulation during application of diphenhydramine for 60 sec were compared with those before the drug application. A retaining current of 10–20 nA was applied to prevent the spontaneous release of diphenhydramine and glutamate from the pipette. The spikes elicited by vestibular nerve stimulation were amplified and displayed on an oscilloscope (Nihon Kohden, VC-9). Ten to 40 successive responses were photographed and simultaneously stored on a magnetic tape. Statistical significance of the data was determined by the Student’s t-test. After the termination of each experiment, the recording sites were marked by passing a direct current of 20–50 μA for 15–25 sec, and histologically confirmed with cresyl violet stain. Further details of procedures were reported previously (4–6).

Results

Spike generation of MVN and LVN neurons: The field potentials in the MVN and LVN with vestibular nerve stimulation consisted of P, N1 and N2 waves, as already designated by Shimazu and Precht (7). The single neurons in the MVN and LVN were classified into two types according to the firing pattern and latency of the first spike elicited by vestibular nerve stimulation. The first type, monosynaptic neurons, fired spikes on the N1 wave of the field potential with a consistent latency of less than 1.6 msec (Figs. 1A and 2A). In the second type, regarded as polysynaptic neurons, the first spike was produced on the N2 wave of the field potential with relatively dispersed latencies ranging from 2.0–3.4 msec (Figs. 1D and 2D). The mean spike latencies of 22 MVN and 29 LVN monosynaptic neurons upon vestibular nerve stimulation were 1.20±0.07 (S.E.) and 1.25±0.05 msec, respectively; and those of 17 MVN and 12 LVN polysynaptic neurons were 2.60±0.11 and 2.71±0.15 msec, respectively (Table 1).

When glutamate up to 100 nA was iontophoretically applied to these neurons for 20–30 sec, the spontaneous firing rate was markedly increased. Therefore, it was considered that the drug in the pipette was properly ejected to the immediate vicinity of
Fig. 1. Effects of iontophoretic application of diphenhydramine (DP) on spikes of monosynaptic (A–C) and polysynaptic (D–F) neurons in the medial vestibular nucleus upon vestibular nerve stimulation. A and D: before, B and E: during 100 nA of DP, and C and F: during 200 nA of DP. P, N, and N2 waves of field potential are simultaneously recorded upon nerve stimulation. Solid triangles indicate stimulus artifacts. Calibration: 5 msec, 0.5 mV.

Table 1. Effects of diphenhydramine (DP) on spike generation of medial vestibular nucleus (MVN) and lateral vestibular nucleus (LVN) upon vestibular nerve stimulation

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>DP 100 nA</th>
<th>DP 200 nA</th>
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</thead>
<tbody>
<tr>
<td><strong>MVN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monosynaptic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurons (22)*</td>
<td>1.26±0.14</td>
<td>1.04±0.13</td>
<td>0.83±0.08*</td>
</tr>
<tr>
<td>Latency (msec)</td>
<td>1.20±0.07</td>
<td>1.20±0.07</td>
<td>1.22±0.08</td>
</tr>
<tr>
<td>Polysynaptic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurons (17)</td>
<td>1.05±0.10</td>
<td>0.80±0.07**</td>
<td>0.59±0.09**</td>
</tr>
<tr>
<td>Latency (msec)</td>
<td>2.60±0.11</td>
<td>2.70±0.12</td>
<td>2.80±0.14</td>
</tr>
<tr>
<td><strong>LVN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monosynaptic</td>
<td></td>
<td></td>
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<tr>
<td>Neurons (29)</td>
<td>1.46±0.12</td>
<td>1.38±0.11</td>
<td>1.33±0.12</td>
</tr>
<tr>
<td>Latency (msec)</td>
<td>1.25±0.05</td>
<td>1.32±0.06</td>
<td>1.31±0.06</td>
</tr>
<tr>
<td>Polysynaptic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurons (12)</td>
<td>1.04±0.10</td>
<td>0.68±0.16</td>
<td>0.51±0.12**</td>
</tr>
<tr>
<td>Latency (msec)</td>
<td>2.71±0.15</td>
<td>2.90±0.19</td>
<td>3.05±0.25</td>
</tr>
</tbody>
</table>

Each value represents the mean±standard error. *( ) indicates the number of neurons tested. Significant difference at *P<0.05 and at **P<0.01, as compared with the value before DP application.

the target neuron. It was also confirmed in the respective neuron that the current effect was negligible by applying Na+ up to 200 nA.

Effects of diphenhydramine on MVN neurons: Figure 1 shows the typical effects of diphenhydramine on spike generation of the MVN monosynaptic and polysynaptic neurons elicited by vestibular nerve stimulation. The monosynaptic neuron was not affected by iontophoretic application of diphenhydramine up to 100 nA, while it was inhibited with 200 nA of the drug (Fig. 1B and C). The mean spike number of 22 MVN monosynaptic neurons was significantly (P<0.05) reduced to 0.83±0.08 (S.E.) with 200 nA of diphenhydramine from 1.26±0.14 before the drug administration (Table 1). The mean spike latency was not affected by
diphenhydramine up to 200 nA.

In contrast to the monosynaptic neuron, spike generation of the MVN polysynaptic neuron upon vestibular nerve stimulation was dose-dependently inhibited by iontophoretic application of diphenhydramine, as demonstrated in Fig. 1E and F. The mean spike number of 17 polysynaptic neurons was 1.05±0.10 before the drug application, while the number was significantly (P<0.05 or P<0.01) decreased to 0.80±0.07 and 0.59±0.09 during diphenhydramine ejection at 100 and 200 nA, respectively (Table 1). The spike latency was only slightly affected by application of diphenhydramine.

Effects of diphenhydramine on LVN neurons: Figure 2 illustrates the typical effects of diphenhydramine on spike generation of monosynaptic and polysynaptic neurons in the LVN. The spike generation of monosynaptic neuron upon vestibular nerve stimulation remained unaffected with iontophoretic application of the drug up to 200 nA (Fig. 2B and C). The mean spike numbers of 29 LVN monosynaptic neurons were 1.46±0.12 and 1.33±0.12 before and during the application of 200 nA, respectively (Table 1).

In the LVN polysynaptic neuron, the spike generation was not affected by 100 nA of diphenhydramine. When the dose increased to 200 nA, a significant (P<0.01) reduction in the spike number was observed in the polysynaptic neurons, and the mean number of 12 neurons tested was decreased to 0.51±0.12 from 1.04±0.10; whereas the mean latency was only slightly affected (Table 1).

Discussion

Vestibular nucleus neurons have been classified into two types: kinetic and tonic neurons (7, 8). The kinetic neuron fired spikes on the N1 wave (monosynaptic component) of the field potential with a consistent latency upon vestibular nerve stimulation. Spontaneous firing of the kinetic neuron rapidly increased in response to an angular acceleration of the horizontal rotation and decreased during a constant angular velocity. The tonic neuron fired spikes on the N2 wave (polysynaptic component) of the field potential with a relatively dispersed latency upon vestibular nerve stimulation. The spontaneous firing rate of the tonic neuron showed a relatively regular pattern, and it initially increased with horizontal rotation followed by a constant rate during the remainder of constant acceleration. As previously discussed (5), the monosynaptic and polysynaptic neurons in the present study were regarded as the kinetic and tonic neurons, respectively.

It was found that the spike generation of MVN polysynaptic neurons upon vestibular nerve stimulation was dose-dependently and most effectively inhibited with iontophoretic application of diphenhydramine in this study, and the MVN monosynaptic neuron was also suppressed with the highest dose (200 nA) used. In contrast to the MVN neurons, diphenhydramine did not affect the spike generation of the monosynaptic neuron in the LVN, although that of the LVN polysynaptic neuron was inhibited with 200 nA of the drug. These effects of diphenhydramine were in contrast to those of ethanol, which inhibited the LVN monosynaptic neuron without affecting the LVN polysynaptic neuron (5, 9). The major MVN neurons send their axons to the abducens nucleus via the ascending medial longitudinal fasciculus and the minor MVN neurons to the spinal cord via the lateral vestibulospinal tract. The LVN neurons mainly send the axons to the spinal cord via the lateral vestibulospinal tract and to the reticular nuclei (10). Since high frequency stimulation of the vestibular nerve produced nystagmus (11), it is conceivable that massive impulses from the peripheral labyrinth to the abducens nucleus
relying at the MVN induce nystagmus, thereby resulting in the production of dizziness and/or autonomic syndromes such as emesis. Therefore, our results herein suggest that the prevention by diphenhydramine of motion sickness without accompanying ataxia is mainly due to an inhibition of transmission in the MVN neurons, particularly in the polysynaptic neurons of the MVN.

The effects of diphenhydramine on the MVN neurons are not considered to be due to a local anesthetic action because the size of the spikes elicited by vestibular nerve stimulation remained unaltered during iontophoretic application of the drug, although the spike number was reduced (cf. Fig. 1). It is also unlikely that the inhibitory effect of diphenhydramine on the MVN neurons were due to anticholinergic action since the drug did not affect the synaptic transmission in the LVN monosynaptic neurons where acetylcholine is probably involved in this transmission, as reported previously (6). Iontophoretically applied histamine reportedly inhibited a majority of the MVN and LVN neurons, and the histamine-induced inhibition was antagonized by metiamide, a H2-receptor blocking agent, but not by diphenhydramine, a H1-receptor blocking agent (12). Taking this observation into account, the effects of diphenhydramine on the vestibular nuclei obtained herein are probably not mediated by the histamine receptor.

References


