Effects of Anti-Vertigo Drugs on Medial Vestibular Nucleus Neurons Activated by Horizontal Rotation

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ABSTRACT — The effects of anti-vertigo drugs on medial vestibular nucleus (MVN) neurons were examined to assess the site and mode of action using cats anesthetized with α-chloralose. Single neuron activity in the MVN was extracellularly recorded using a silver wire microelectrode attached along a seven-barreled micropipette, each of which was filled with diphenhydramine, diphenidol, betahistine, glutamate or NaCl. Type I of the MVN neurons were identified according to the responses obtained when the animal placed on a turn-table was rotated sinusoidally. The effects of the drugs were examined on type I neurons which received impulses primarily from the labyrinth and sent them to the oculomotor nuclei. The microiontophoretic application of diphenhydramine, diphenidol and betahistine inhibited rotation-induced firing of type I MVN neurons. Diphenhydramine and diphenidol were more potent than betahistine. These results suggest that these drugs directly act on MVN neurons to reduce the responsiveness to rotatory stimulation.

Diphenidol and betahistine, structurally related to histamine, are known to be anti-vertigo drugs and are used for the treatment of vertigo and dizziness in such diseases as Ménière's disease (1, 2). Diphenhydramine is widely used to prevent motion sickness. Our previous studies showed that the microiontophoretic application of betahistine and diphenhydramine inhibited the spike generation of polysynaptic neurons in the lateral vestibular nucleus (LVN) elicited by vestibular nerve stimulation (3, 4). In addition, the inhibition by diphenidol of spike generation in the vestibular nucleus neuron with vestibular nerve stimulation has also been reported by Matsuoka et al. (5).

The LVN neurons, which receive input from the peripheral vestibule and project mainly to the spinal cord, are involved in the vestibulo-spinal reflex, although some also project to the oculomotor and abducens nuclei. On the other hand, the MVN neurons send axons mainly to the abducens nuclei and are involved in the vestibulo-ocular reflex, although some project to the spinal cord. However, it is not known whether or not these anti-vertigo drugs selectively act on the medial vestibular nucleus (MVN) neurons (6–8).

Therefore, a microiontophoretic study was undertaken to determine whether or not these anti-vertigo drugs act on the MVN neurons, which receive input from the horizontal semicircular canal and are involved in the vestibulo-ocular reflex.

MATERIALS AND METHODS

Fifteen male adult cats weighing 3.2–4.5 kg were used. After cannulation into the trachea and femoral veins under ether anesthesia, the ether was replaced with α-chloralose (30 mg/kg, i.v.), with supplemental doses at 10
mg/kg. All pressure points and wounds were locally anesthetized with 8% lidocaine repeatedly throughout the experiment. The animal was fixed in a stereotaxic apparatus with its head inclined 15° nose-down on the ear bar axis to optimally isolate horizontal canal stimulation. Then, the animal was placed on a turntable that was manually rotated sinusoidally in a horizontal plane at an angular velocity of ca. 60°/sec for 120°. Body temperature was maintained at 36.5–37.5°C with a heating pad.

Extracellular neuron activities in the MVN (P:8 to 8.5, L:2.5 to 3.0, H:−3 to −3.5) (9) were recorded using a glass-insulated silver wire microelectrode (electrical resistance: approx. 1 MΩ) attached along a seven-barreled micropipette. The outer diameter of the micropipette was 7–8 μm, and the distance between the tips of the recording electrode and the micropipette was 30–50 μm. The neuronal activities displayed on an oscilloscope (Nihon Kohden, VC-11) were recorded on a recticorder (Nihon Kohden, RJG-4124) via a spike counter (DIA Medical System, DSE-235P). Each micropipette was filled with 100 mM diphenhydramine HCl (Kowa Co.), 25 mM diphenidol (Nippon Shinyaku Co.), 100 mM betahistine mesylate (Eisai Co.), 1 M monosodium L-glutamate (Wako Co.) or 3 M NaCl. These chemicals were iontophoretically applied in the immediate vicinity of the target neuron being recorded using a microiontophoresis programmer (WP-I, Model 160). All chemicals tested were ejected as cations except for glutamate which was given as an anion.

After the termination of each experiment, the recording site was marked by passing a direct current of 20 μA for 10–20 sec through the electrode and histologically checked by staining with cresyl violet.

The means of the maximum firing rates were obtained from the last five trial rotations of the turntable out of 6 rotations. Then the statistical significance between the means before and during application of the drug in each neuron was determined using Student's t-test. Furthermore, the mean of the maximum firing rate during application of the drug was expressed as a percentage of the control. The statistical significance between the means (%) of all neurons tested before and during the drug application was also determined by an unpaired Student's t-test. Percent changes in the maximum firing rate by rotation during application of the drug were obtained in each neuron as 100% before administration of the drug, and the means of the neurons tested at doses of 100 and 200 nA were also calculated. Further details of the experimental procedures have been described elsewhere (10).

RESULTS

MVN neurons were classified into four types according to their responses to horizontal sinusoidal rotation as described by Duensing and Schaefer and in our previous study (10, 11). Briefly, type I neurons exhibited an increase in firing with a horizontal rotation directed ipsilateral to its recording site and a decrease with contralateral rotation, whereas type II neurons showed opposite responses (Fig. 1B). Type III and type IV exhibited only an increase and a decrease in firing with horizontal sinusoidal rotations, respectively. The effects of the drugs were tested only on type I neurons. Out of 41 neurons being recorded, 26, 12, 2 and 1 neurons were identified as type I, II, III and IV neurons, respectively. The effects of the drugs were tested only on type I neurons. Out of 41 neurons being recorded, 26, 12, 2 and 1 neurons were identified as type I, II, III and IV neurons, respectively. A histological study revealed that, 22 out of the 26 type I neurons were located in the MVN. As Lannou et al. described, type I neurons receive input from the labyrinth and project to the abducens nuclei on ipsilateral and contralateral sites, whereas type II neurons showed opposite responses (Fig. 1B). Type III and type IV exhibited only an increase and a decrease in firing with horizontal sinusoidal rotations, respectively. The effects of the drugs were tested only on type I neurons. Out of 41 neurons being recorded, 26, 12, 2 and 1 neurons were identified as type I, II, III and IV neurons, respectively. A histological study revealed that, 22 out of the 26 type I neurons were located in the MVN. As Lannou et al. described, type I neurons receive input from the labyrinth and project to the abducens nuclei on ipsilateral and contralateral sites, whereas type II neurons show opposite responses (Fig. 1B). Type III and type IV exhibited only an increase and a decrease in firing with horizontal sinusoidal rotations, respectively. The effects of the drugs were tested only on type I neurons. Out of 41 neurons being recorded, 26, 12, 2 and 1 neurons were identified as type I, II, III and IV neurons, respectively. A histological study revealed that, 22 out of the 26 type I neurons were located in the MVN. As Lannou et al. described, type I neurons receive input from the labyrinth and project to the abducens nuclei on ipsilateral and contralateral sites, whereas type II neurons receive input from the collateral axon of the type I neurons which project to the contralateral sites and inhibit type I neurons on the ipsilateral sites (12). Therefore, in this study, the effects of drugs were examined on the 22 type I neurons to evaluate their action on the main pathway involved in the vestibulo-ocular reflex.

When diphenhydramine at doses of 100 and
200 nA was iontophoretically applied to 6 and 5 neurons, respectively, the increase in firing induced by horizontal rotation was significantly (P < 0.01) reduced in 4 and 5 neurons, respectively (Fig. 1A, Table 1). In these affected neurons, spontaneous firing and glutamate-induced firing also decreased during iontophoretic application of the drug, but the amplitude and form of the spike were not affected. Complete recovery was usually seen 10–20 min after cessation of the drug application. In addition, the means of the maximum firing rates were also significantly (P < 0.01) reduced to 69.3 ± 6.5 and 14.4 ± 6.6% of the control during the microiontophoretic application of diphenhydramine at doses of 100 and 200 nA, respectively (Table 1).

The iontophoretic application of diphenidol at doses of 100 and 200 nA significantly (P < 0.01) reduced the increase in firing induced by the horizontal rotation in 4 of 9 and 8 of 9 neurons tested, respectively (Fig. 2, Table 2).

![Fig. 1. Effect of iontophoretically applied diphenhydramine on the rotation- and glutamate-induced firing of medial vestibular nucleus neurons (A), and firing pattern of type I (a) and type II (b) neurons in the medial vestibular nucleus (B).](image)

**Fig. 1.** Effect of iontophoretically applied diphenhydramine on the rotation- and glutamate-induced firing of medial vestibular nucleus neurons (A), and firing pattern of type I (a) and type II (b) neurons in the medial vestibular nucleus (B).

**Table 1.** Effect of iontophoretically applied diphenhydramine (DPH) on medial vestibular nucleus neurons

| Neuron No. | Control | DPH 100 nA (%) | ( %)* | DPH 200 nA (%) | ( %)* | 3 min⁴ | (%)⁴ |
|------------|---------|----------------|-------|----------------|-------|--------|-------|
| 1          | 32.8    | 21.1**         | (64.3)| 3.8**          | (11.6)| 27.1   | (82.6)|
| 2          | 53.8    | 25.4**         | (47.2)|               |       | 42.8   | (79.6)|
| 3          | 53.6    | 39.3**         | (73.3)| 2.9**          | (5.4)| 27.1   | (50.6)|
| 4          | 137.7   | 132.3          | (96.1)| 6.2**          | (4.5)| 153.1  | (111.2)|
| 5          | 122.3   | 85.4**         | (69.8)| 49.2**         | (40.2)| 94.6   | (77.4)|
| 6          | 80.0    | 52.1*          | (65.1)| 8.2**          | (10.3)| 65.6   | (82.0)|

Mean ± S.E. 80.0 ± 17.1 59.4 ± 17.4 (69.3 ± 6.5***) 14.1 ± 8.8 (14.4 ± 6.6**) 68.4 ± 19.9 (80.5 ± 7.9)

Each value represents the firing rate/sec. *: The values in parentheses are represented as percentages of the respective controls. #: 3 to 15 min after cessation of drug application. *: P < 0.05, **: P < 0.01.
In addition, the means of the maximum firing rate with the rotation were also significantly (P < 0.01) reduced to 73.7 ± 3.5 and 28.0 ± 8.6% with diphenidol at 100 and 200 nA, respectively (Table 2). Diphenidol at 100 and 200 nA also inhibited spontaneous and glutamate-induced firing to less than 50% of the control in all 9 neurons tested without affecting the amplitude or form of the spike. The effects of diphenidol were long lasting, and complete recovery was usually seen in 15 – 30 min.

Betahistin was iontophoretically applied to 7 neurons at a dose of 100 nA and 6 neurons at 200 nA, and there was a significant (P < 0.01) reduction in the increase in firing induced by horizontal rotation in 2 and 4 neurons, respectively (Fig. 3, Table 3). In addi-

![Fig. 2. Effect of iontophoretically applied diphenidol on the rotation- and glutamate-induced firing of medial vestibular nucleus neurons.](image)

| Neuron No. | Control | DPD 100 nA | (%)a | DPD 200 nA | (%)a | 3 minb | (%)a |
|------------|---------|------------|------|------------|------|--------|------|
| 1          | 31.3    | 22.8**     | (72.9)| 0.3**      | (1.0)| 22.3   | (71.4)|
| 2          | 17.9    | 13.6*      | (75.9)| 3.4**      | (19.0)| 11.7   | (65.5)|
| 3          | 47.7    | 28.1**     | (59.0)| 6.8**      | (14.3)| 25.4   | (53.2)|
| 4          | 29.3    | 24.2       | (82.7)| 23.0       | (78.5)| 29.9   | (102.0)|
| 5          | 53.6    | 38.2**     | (71.3)| 31.8**     | (59.3)| 41.8   | (78.0)|
| 6          | 26.4    | 15.4*      | (58.3)| 9.3**      | (35.2)| 25.7   | (97.3)|
| 7          | 153.1   | 107.7**    | (70.4)| 34.6**     | (22.6)| 113.1  | (73.9)|
| 8          | 113.8   | 96.2       | (84.5)| 1.5**      | (1.3)| 122.3  | (107.5)|
| 9          | 109.2   | 96.7       | (88.6)| 22.8**     | (20.9)| 80.0   | (73.3)|

Mean±S.E. 64.7±16.1 49.2±13.0 (73.7±3.5**) 14.8±4.4 (28.0±8.6**) 52.5±13.9 (80.2±6.0**)  

Each value represents the firing rate/sec. a: The values in parentheses are represented as percentages of the respective controls. b: 3 to 15 min after cessation of drug application. *: P < 0.05. **: P < 0.01.
Fig. 3. Effect of iontophoretically applied betahistine on the rotation-induced firing of medial vestibular nucleus neurons.

Table 3. Effect of iontophoretically applied betahistine (BH) on medial vestibular nucleus neurons

| Neuron No. | Control | BH 100 nA (%) | BH 200 nA (%) | 3 min (%) | Mean ± S.E. | 3 min (%) |
|------------|---------|---------------|---------------|-----------|-------------|-----------|
| 1          | 72.6    | 39.3**        | 19.3**        | 62.6      | 54.4±12.3   | 86.2      |
| 2          | 59.1    | 51.4*         | 33.7**        | 60.5      | 42.0±9.6    | 102.4     |
| 3          | 74.8    | 56.8**        | 14.3**        | 70.8      | 79.1±5.0**  | 94.7      |
| 4          | 24.9    | 24.0          | 14.3**        | 25.3      | 56.5±7.0**  | 102.1     |
| 5          | 14.9    | 11.1*         | 10.5**        | 17.9      | 28.7±10.6   | 120.1     |
| 6          | 104.9   | 86.7          | 79.0*         | 115.3     | 54.6±12.8   | 109.9     |
| 7          | 29.3    | 24.4          | 15.3*         | 29.6      | 75.9±5.0**  | 101.0     |

Each value represents the firing rate/sec. *: The values in parentheses are represented as percentages of the respective controls. **: 3 to 15 min after cessation of drug application. *: P < 0.05, **: P < 0.01.

The means of the maximum firing rate were also significantly (P < 0.01) reduced to 79.1 ± 5.0 and 56.5 ± 7.0% of the control, with microiontophoretic application of betahistine at 100 and 200 nA, respectively (Table 3). However, the spontaneous firing rate as well as the spike amplitude was rarely affected by betahistine up to 200 nA.

DISCUSSION

Diphenhydramine has been reported to inhibit the spontaneous firing of MVN neurons, although the neurons were not identified (13). Previously, we found that this drug predominantly inhibited the synaptic transmission in LVN neurons, which were polysynaptically activated by vestibular nerve stimulation (4). In the present study, it was found that the microiontophoretic application of the drug inhibited an increase in firing of type I neurons induced by ipsilateral horizontal rotation. The results are in agreement with those obtained by Kirsten and Sharma (14). The systemic and microiontophoretic applications of betahistine also inhibited the LVN neurons polysynaptically activated by electrical stimulation, but not those excited monosynaptically (3). Similarly, the systemic injection of diphenidol inhibited spike generation by vestibular nerve stimulation in LVN neurons (5). In line with these results, the present study revealed that the microiontophoretic application of betahistine and diphenidol also inhibited the re-
sponses to horizontal rotation in the MVN.

In these previous studies, diphenhydramine and betahistine inhibited the LVN neurons activated polysynaptically, but not monosynaptically, by the vestibular nerve stimulation. The interneurons in the LVN, probably including both neurons projecting to the spinal cord and the nuclei related with eye movements, were considered to be affected by these anti-vertigo drugs since the vestibular nerve contains both afferents from the semicircular canals and otolith (3, 4). However, the finding that the MVN type I neurons activated by the horizontal rotation were inhibited by these drugs herein indicates that the neurons receiving input from the semicircular canal of the vestibule are affected by these drugs. Since type I MVN neurons mainly innervate the bilateral abducens nuclei (6–8), our results suggest that these anti-vertigo drugs inhibit MVN neurons involved in the vestibulo-ocular reflex.

The order of potency of inhibiting the rotation-induced increase in firing of the type I neurons was: diphenidol with long-lasting effects and diphenhydramine and betahistine, which had relatively selective effects on the rotation-induced response.

In conclusion, it seems useful in evaluating anti-vertigo drugs to examine whether or not the drugs affect the responses of the MVN neurons to horizontal rotation.

REFERENCES

1 Bertrand, R.A.: Modification of the vestibular function with betahistine HCl. Laryngoscope 81, 889–898 (1971)
2 Elia, J.C.: Double-blind evaluation of a new treatment for Ménière's syndrome. JAMA 196, 187–189 (1966)
3 Unemoto, H., Sasa, M., Takaori, S., Ito, J. and Matsuoka, I.: Inhibitory effect of betahistine on polysynaptic neurons in the lateral vestibular nu-

3ceus. Arch. Otorhinolaryngol. 236, 229–236 (1982)
4 Takatani, T., Ito, J., Matsuoka, I., Sasa, M. and Takaori, S.: Effects of diphenhydramine iontophoretically applied onto neurons in the medial and lateral vestibular nuclei. Japan. J. Pharmacol. 33, 557–561 (1983)
5 Matsuoka, I., Takaori, S. and Morimoto, M.: Effects of diphenidol on the central vestibular and visual systems of cats. Japan. J. Pharmacol. 22, 817–825 (1972)
6 Gacek, R.R.: Vestibular neuroanatomy recent observations. Ann. Otol. 88, 667–675 (1979)
7 Shimazu, H.: Neuronal organization of the pre-motor system controlling horizontal conjugate eye movements and vestibular nystagmus. In Motor Control Mechanisms in Health and Disease, Edited by Desmedt, J.E., Raven Press, New York (1983)
8 Carleton, S.C. and Carpenter, M.B.: Afferent and efferent connections of the medial, inferior and lateral vestibular nuclei in the cat and monkey. Brain Res. 278, 29–51 (1983)
9 Snider, R.S. and Niemer, W.T.: A Stereotaxic Atlas of the Cat Brain. Univ. of Chicago Press, Chicago (1981)
10 Nakamura, J., Sasa, M. and Takaori, S.: Ethanol potentiates the effect of γ-aminobutyric acid on medial vestibular nucleus neurons responding to horizontal rotation. Life Sci. 45, 971–978 (1989)
11 Duensing, F. and Schaefer, K.P.: Die Aktivität einzelner Neurone im Bereich der Vestibularis-kerne bei Horizontalbeschleunigungen unter be-
sonderer Berücksichtigung des vestibulären Nys-
tagmus. Arch. Psychiat. Nervenkr. 198, 225–252 (1958)
12 Lannou, J.W., Precht, W. and Cazin, L.: The postnatal development of functional properties of central vestibular neurons in the rat. Brain Res. 175, 219–232 (1979)
13 Sekitani, T., McCabe, B.F. and Ryu, J.H.: Drug effects on the medial vestibular nucleus. Arch. Otolaryngol. 93, 581–589 (1971)
14 Kirsten, E.B. and Sharma, J.N.: Microiontophoresis of acetylcholine, histamine and their antagonists on neurons in the medial and lateral vestibular nuclei of the cat. Neuropharmacology 15, 743–753 (1976)