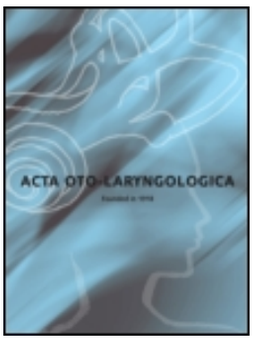

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Effect of Experimental Acidosis on Nystagmus in Rabbits

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Vertigo related to acidosis in Meniere's disease has been reported. This study was undertaken to ascertain whether acidosis has any effect on vertigo. Since patients with Meniere's disease usually show unilateral vestibular dysfunction, unilateral intratympanic injection of streptomycin sulfate (SM) was used to induce unilateral vestibular dysfunction in rabbits. Intratympanic SM injections induced vestibular destruction and elicited severe spontaneous nystagmus and ataxia. Then symptoms of acute vestibular upset gradually subsided and eventually disappeared completely. Three weeks after SM injections, in compensated rabbits, NH_4Cl injection or CO_2 inhalation was used to induce acidosis. Intravenous NH_4Cl injection or CO_2 inhalation induced nystagmus and ataxia again. In normal rabbits, no nystagmus was induced by NH_4Cl injection or by CO_2 inhalation. These results suggest that acidosis might be a cause of recurrence of vertigo in patients with unilateral vestibular dysfunction. *Key words*: *unilateral vestibular dysfunction, NH_4Cl, CO_2, recurrence of vertigo.*

INTRODUCTION

Vertiginous attack associated with acidosis in Meniere's disease has been reported (1–4). Takahashi et al. (1) described 3 cases of Meniere's disease. During the vertiginous attacks, all 3 cases had metabolic acidosis and 2 of them had also respiratory acidosis (1). Makimoto (2) reported 3 cases with metabolic acidosis and Cellestino et al. (4) observed 4 cases with metabolic acidosis in 10 and 55 cases of Meniere's disease, respectively. Although the indicence of acidosis in vertiginous attacks has been reported to be relatively low in Meniere's disease, elucidation of the correlation between acidosis and vertiginous attacks may shed some light on the mechanism of vertigo in Meniere's disease.

Administration of NH_4Cl is known to induce metabolic acidosis, and CO_2 inhalation to induce respiratory acidosis (5). In this study, the effects of NH_4Cl injection or CO_2 inhalation on eye movements and posture in rabbits with unilateral vestibular dysfunction were examined.

MATERIAL AND METHODS

Preparation of rabbits with unilateral vestibular dysfunction

Fifty-eight adult pigmented rabbits with black eyes (Dutch Ireland) of both sexes (1.5–2.5 kg) with normal eardrums, showing no spontaneous nystagmus and showing normal postrotatory nystagmus, were prepared. Twenty-eight rabbits were used for normal controls and 30 rabbits were injected with streptomycin sulfate (SM) into the right middle ear through the tympanic membrane. Usually, two to three injections of SM daily (0.6 g/day) dissolved in 3 ml of physiological saline were enough to induce unilateral vestibular dysfunction. Rabbits with vigorous spontaneous nystagmus (fast phase to the left side) induced by SM were used. These rabbits also showed head turning toward the right and longitudinal axis body rolling (6). Nystagmus gradually subsided in a few days. Spiral rotation of the body towards the right side, hypertonic and extended left legs and hypotonic right legs persisted unchanged. When caloric tests using 50 ml of ice water were performed, nystagmus was not observed in the right ear (7).

Measurements of arterial pH and pCO_2

The arterial blood pH and pCO_2 were analyzed with ABL-2 blood gas analyzer (Shinkoh Koueki, Tokyo). Throughout the experiments, the arterial blood was collected anaerobically and kept cool in ice before analysis. Results were corrected by the body temperatures (8). Rabbits exhibited vigorous nystagmus, head turning and rolling movements one day after SM injections, and the arterial blood pH of 8 rabbits was measured (group I). As a control, arterial blood pH was also checked in 28 normal rabbits (group II). The results were compared using the Student's unpaired two-tailed t-test.

Measurements of eye movements

Three weeks after SM injections, nystagmus disappeared completely. Therefore, all experiments were carried out 3 weeks after SM injections. Rabbits were fixed with stainless steel frames molded according to the shape of the body. This frame did not allow any major body or leg movements, but did not cause discomforting pressure. The head was fixed with a collar and a mouthpiece. Eye movements were recorded from the canthi of the left eye with the Rectigraph 144 electronystagmograph (Sanei, Tokyo). The time constants for recording the eye movements were 3.0 and 0.03 s.

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Induction of metabolic acidosis with NH_4Cl injection

0.25 M NH_4Cl in 20 ml of physiological saline was injected intravenously for 30 s in 9 SM-injected rabbits. As controls, physiological saline (20 ml) was injected intravenously for 30 s in 5 SM-injected rabbits. 0.25 M NH_4Cl in 20 ml of physiological saline was also injected intravenously in 5 normal rabbits. Physiological saline (20 ml) was injected intravenously in 3 normal rabbits.

Induction of respiratory acidosis with CO_2 inhalation

Eight SM-injected rabbits were placed in the frame in a closed chamber ($70 \times 40 \times 40$ cm) and 6 rabbits received CO_2 gas for 10 min at a CO_2 concentration of approximately 7% in air. Remaining 2 rabbits were placed in a closed chamber but received no CO_2 gas. Three normal rabbits also received the same amount of CO_2 gas for 10 min and two normal rabbits were placed in a closed chamber but received no CO_2 gas. Arterial blood pH and gas were analyzed as described above. The eye movements were also recorded with the electronystagmograph.

Preparation of specimens

After the above experiments all rabbits were perfused with normal saline followed by a 10% buffered formaldehyde fixative under pentobarbital anesthesia, after which both ears were removed and kept in an immersion fixation of 10% buffered formaldehyde. The specimens were decalcified in 5% trichloroacetic acid, dehydrated, and embedded using the celloidine-paraffin method. Hematoxylin-eosin stained serial sections were made of the temporal bones.

RESULTS

Effect of SM-induced vertigo on arterial blood pH

The arterial blood pH of 8 rabbits with vigorous spontaneous nystagmus (1 day after SM injection) (group I) was compared with that of 28 normal rabbits (group II). Mean arterial blood pH ($\pm$ S.E.M.) was 7.33 ($\pm$ 0.07) for group I and 7.36 ($\pm$ 0.07) for group II. Using the two-tailed unpaired t-test there was no significant difference in blood pH between these two groups.

Effects of NH_4Cl injection on eye movements and posture

Nine rabbits, in which nystagmus by SM had completely disappeared 3 weeks after SM injections, received intravenous injection of NH_4Cl. Soon after NH_4Cl injection (0–10 s, average 3.1 s), spontaneous nystagmus was observed in all 9 rabbits (Table I). Nystagmus occurred with rapid component to the left in 2 rabbits, to the right in 3, and changing from the left to the right in 4. Nystagmus to the left continued for a short time (60 and 90 s), then disappeared. Nystagmus to the right had a longer duration (0.5–12.5 min, average 6.9 min) than nystagmus to the left. In nystagmus changing from the left to the right, nystagmus to the left also continued for a very short time (10–30 s, average 16.7 s), then changed to the right and continued for a longer period (4.5–35.5 min, average 16.6 min). A typical pattern of the nystagmus induced by NH_4Cl injection is shown in Fig. 1. When the rabbits were released from the frames, body rolling became marked. Two of the 9

Table I. *Effects of experimental acidosis on induced nystagmus*

	Treatment	No. of rabbits tested	No. of rabbits showing nystagmus	Latent period of nystagmus (average)	Duration of nystagmus (average)	Direction of nystagmus
Streptomycin injected rabbits						
	NH_4Cl injected	9	9	0 ~ 10 sec (3.1 sec)	0.5 ~ 36 min (9.6 min)	Left 2, Right 3 From left to right 4
	Saline injected	5	0*	—	—	—
	CO_2 inhaled	6+	5	0 ~ 40 sec (15 sec)	1.8 ~ 7.2 min (4.2 min)	Right 5
	No CO_2 inhaled	2	0*	—	—	—
Normal rabbits						
	NH_4Cl injected	5	0*	—	—	—
	Saline injected	3	0*	—	—	—
	CO_2 inhaled	3	0*	—	—	—
	No CO_2 inhaled	2	0*	—	—	—

* No nystagmus was observed during 1 h after treatment.
\+ One rabbit died during CO_2 inhalation.

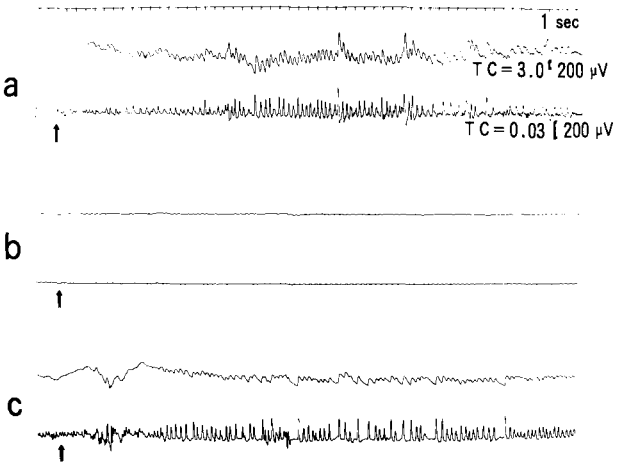

Fig. 1. Typical pattern of nystagmus. Electrodes were placed on both sides of the left eye to record the horizontal component, and another electrode was placed on the forehead. *TC*, time constant. In *b* and *c*, the recordings were done under the same conditions, concerning TC (upper recording, TC = 3.0; lower recording, TC = 0.03) and voltage as shown in *a*. In rabbits in which SM-induced nystagmus had completely disappeared, *a*: NH_4Cl intravenous injection induced nystagmus again, *b*: physiological saline intravenous injection induced no nystagmus and *c*: CO_2 inhalation induced nystagmus again. The *arrows* indicate the points at which injection or inhalation ended.

rabbits received NH_4Cl injection twice (2 days later in one rabbit and 3 weeks later in the other rabbit) and this second NH_4Cl injection induced nystagmus again in these 2 rabbits. Before and 15 min after NH_4Cl injection, arterial blood pH was measured in 2 rabbits showing nystagmus. Arterial blood pH changed from 7.41 to 7.16 in one rabbit and from 7.46 to 6.97 in the other rabbit. As a control, arterial blood pH was measured in 5 normal rabbits to examine the influence of NH_4Cl injection. The mean arterial blood pH before NH_4Cl injection was 7.34 ± 0.09. After NH_4Cl injection, all the rabbits showed sudden decrease of arterial blood pH (7.09 ± 0.07) which recovered to normal level within about 30 min. None of the normal rabbits showed nystagmus after intravenous injection of NH_4Cl. Intravenous injection of physiological saline induced no nystagmus in 5 SM-injected rabbits nor in 3 normal rabbits. The arterial blood pH before and after saline injection did not change significantly in these rabbits. After freeing those rabbits that showed no nystagmus from the frames, no posture changing was observed.

Effects of CO_2 inhalation on eye movements and posture

Six rabbits, in which nystagmus by SM had completely disappeared 3 weeks after SM injections, inhaled CO_2 gas (Table I). Five rabbits showed spontaneous nystagmus to the right. The latent period was from 0 to 40 s (average 15 s). Duration of the nystagmus was from 1.8 min to 7.2 min (average 4.2 min). A typical pattern of the nystagmus induced by CO_2 inhalation is shown in Fig. 1. When freeing the rabbits from the frames, body rolling became marked. Arterial blood gas analysis was carried out before and 10 min after CO_2 inhalation in 3 rabbits showing nystagmus. The means of arterial blood pH and pCO_2 before CO_2 inhalation were 7.40 and 36.87 mmHg, respectively. Ten minutes after CO_2

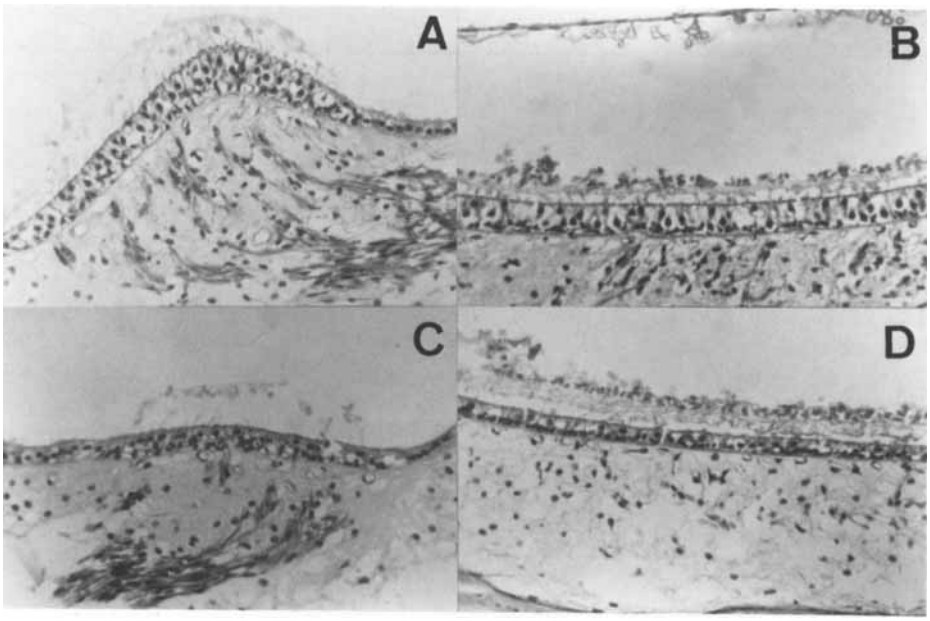

Fig. 2. Photomicrographs demonstrating intact appearance in the crista ampullaris (A) and macula sacculi (B) from the contralateral healthy ear of a rabbit (H & E stain, × 100). Photomicrographs showing near-total loss of hair cells and flattened sensory epithelium in both crista ampullaris (C) and macular sacculi (D) from the ear of a rabbit receiving intratympanic SM injections. (H & E stain, × 100).

inhalation, these changed to 7.01 and 87.97 mmHg, respectively, indicating respiratory acidosis. The procedure was repeated with 2 of these 5 rabbits. The second CO_2 inhalation (1 h later in one rabbit and 1 day later in the other rabbit) induced nystagmus to the right again. Two rabbits which were placed in a closed chamber for 10 min but received no CO_2 gas showed no nystagmus. Although a decrease of arterial blood pH and an increase of pCO_2 were observed after CO_2 inhalation, 3 normal rabbits showed no nystagmus after CO_2 inhalation and 2 normal rabbits, which were placed in a closed chamber for 10 min but received no CO_2 gas, showed no nystagmus. When freeing those rabbits that showed no nystagmus from the frames, no posture changing was observed.

Histological findings

Histopathological study of the ears of rabbits revealed that those rabbits which were injected with SM into the middle ear through the tympanic membrane had loss of hair cells in the cristae and maculae without changes in the supporting elements, as well as flattened sensory epithelium (Fig. 2C and D) in contrast with contralateral healthy ears (Fig. 2A and B).

DISCUSSION

Experimental endolymphatic hydrops has generally been accepted as an animal model of Meniere's disease (9). However, severe postural deviation or spontaneous nystagmus has rarely been observed in this animal model (9). Since patients with Meniere's disease usually have unilateral vestibular dysfunction of various degrees without bilateral involvement (10), rabbits with unilateral vestibular dysfunction were prepared by unilateral injections of SM. SM is an aminoglycoside antibiotic the ototoxicity of which is well know from clinical (11, 12), physiological and histological observations (11, 13–17). SM injections through the tympanic membrane is an easier technique than surgery in inducing experimental vestibular dysfunction. The latter is more harmful to the animals and necessitates general anesthesia (e.g. administration of sodium pentobarbital), which induces respiratory suppression. The rabbits in which severe spontaneous nystagmus and ataxia appeared 1 day after SM injections into the right middle ear through the tympanic membrane were used. Spontaneous nystagmus subsided gradually in a few days and disappeared completely thereafter. The presence of unilateral vestibular dysfunction in such rabbits was confirmed by the caloric test and histopathological observation of the ear revealed vestibular destruction (Fig. 2). Rabbits exhibiting symptoms of acute vestibular upset 1 day after SM injections showed arterial blood pH that was normal. Therefore, acid-base disturbance was found not to participate in vertigo observed 1 day after SM injections.

In acid-base disturbance, the compensation occurs secondarily to normalize blood pH. Hence the experiments were carried out in the acute stage of acidosis in order not to be influenced by the compensation. Intravenous NH_4Cl injection induced spontaneous nystagmus and ataxia again in rabbits in which spontaneous nystagmus induced by SM had completely disappeared 3 weeks after SM injections (Table I and Fig. 1). The arterial blood of these SM-injected rabbits showed acidosis. The duration of the spontaneous nystagmus after NH_4Cl injection (0.5–36 min, average 9.6 min) was nearly as long as that during which the sudden decrease of arterial blood pH after NH_4Cl injection recovered to normal level (about 30 min). As controls, intravenous injection of the same amount of physiological saline to SM injected rabbits did not induce acidosis or nystagmus. In normal rabbits, NH_4Cl injection induced acidosis but no nystagmus. Intravenous injection of the same amount of physiological saline to normal rabbits did not induce acidosis or nystagmus. CO_2 inhalation induced spontaneous nystagmus and ataxia again in rabbits in which spontaneous nystagmus induced by SM had completely disappeared 3 weeks after the SM injections (Table I and Fig. 1). The arterial blood of these rabbits showed acidosis. As controls, these rabbits were kept in the same chamber without CO_2 inhalation for 10 min. This did not induce acidosis or nystagmus. In normal rabbits, CO_2 inhalation induced acidosis, but no nystagmus. Thus, spontaneous nystagmus and ataxia were induced in rabbits with unilateral vestibular dysfunction, but not in normal rabbits, in both metabolic (NH_4Cl injection) and respiratory (CO_2 inhalation) acidosis. These findings indicate that acidosis causes temporary vestibular dehabituation in previously hemilabyrinthectomized rabbits.

There have been few reports on experimental acidosis and nystagmus. Kondo (18) described the influence of CO_2 inhalation on nystagmus observed soon after surgical destruction of the unilateral labyrinth in rabbits. An appropriate dose of CO_2 inhalation increased the frequency and decreased the amplitude of the nystagmus. Unfortunately, he did not observe the effect of CO_2 inhalation in rabbits in which surgically induced nystagmus had disappeared.

Celestino et al. (4) have stated that the water-salt balance of the organism including the acid-base balance has a certain, though still unspecified, influence on the homeostasis of the labyrinthine fluids. Since CO_2 and NH_4Cl can be transported to cerebrospinal fluids (5) and possibly to labyrinthine fluids, acidosis

induced by CO_2 inhalation or NH_4Cl injection may have certain influences on the central and peripheral compensatory functions after unilateral destruction of labyrinths.

It is suggested that acidosis might be a cause of recurrence of vertigo in patients with unilateral vestibular dysfunction. Diabetes mellitus and some renal and thyroid diseases are also known to accompany vertigo or dizziness sometimes (19–21). Acidosis, often seen in these diseases and unilateral vestibular damage, by any cause, will lead to the development of vertiginous attacks in patients with these diseases. Further studies of this problem are necessary.

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