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BEHAVIORAL ASSESSMENT AND IDENTIFICATION OF A MOLECULAR MARKER IN A SALICYLATE-INDUCED TINNITUS IN RATS

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Abstract—Tinnitus is a non-observable phantom sensation. As such, it is a difficult condition to investigate and, to date, no effective treatment has been developed. To approach this phantom sensation, we aimed to develop a rat behavioral model of tinnitus using salicylate, an active component of aspirin known to induce tinnitus. We also aimed to establish a molecular marker of tinnitus by assessing the expression of transient receptor potential cation channel superfamily V-1 (TRPV1) in the rat auditory pathway during salicylate-induced tinnitus. Animals were trained to perform "an active avoidance task": animals were conditioned by electrical footshock to move to the other side of the conditioning box when hearing a sound. Animals received a single injection of saline or salicylate (400 mg/kg i.p.) and false positive responses were measured 2 h after injection as the number of movements during a silent period. The number of responses in salicylate-treated animals was highest when the conditioned stimulus was 60 dB sound pressure level (SPL) and 16 kHz. This indicates that animals could feel tinnitus 2 h after salicylate injection, equivalent to that induced by 60 dB SPL and 16 kHz. By means of real-time PCR and western blot analysis, TRPV1 expression was significantly upregulated in spiral ganglion cells 2 h after salicylate injection and this upregulation together with the increase in the number of false positive responses was significantly suppressed by capsazepine (10 mg/kg i.p.), a specific antagonist of TRPV1. This suggests that salicylate could induce tinnitus through activation of TRPV1 in the rat auditory pathway.

Key words: animal model, TRPV1, spiral ganglion, dorsal cochlea nucleus.

Many people have experienced a sensation of ringing in their ears when no external sound is present. Typically, this sensation of tinnitus is associated with a reversible cause and subsides over a period of time ranging from a few seconds to a few days. However, in 5–15% of the general population, the tinnitus is unremitting (Heller, 2003). Chronic tinnitus is more prevalent among seniors (12% after the age of 60) than in young adults (5% in the age group 20–30), but can occur at any age. In 1–3% of the general population, tinnitus is perceived as loud enough to affect the quality of life (Eggermont and Roberts, 2004). However, no effective therapeutic strategy for such intractable tinnitus has been established.

There are at least two obvious reasons why it is still so hard for clinicians to treat intractable tinnitus. One reason is that, since Jastreboff and Sasaki (1994) proposed an animal behavioral model of tinnitus based on an active avoidance task of drinking water with electrical footshock, better feasibility of the model has been discussed. The first purpose of the present study was, therefore, to develop a rat behavioral model to enable the objective evaluation of tinnitus. Salicylate, an active component of aspirin, is well known to induce an acute and transient type of tinnitus (Cazals, 2000; Eggermont and Roberts, 2004). According to the recent good work of climbing a pole with electrical footshock by Guitton et al. (2003), our model using salicylate was developed with modifications of a much easier active avoidance task. These previous and present tinnitus models will be addressed again in the first paragraph in Discussion.

The other reason is that the molecular mechanism of tinnitus generation in the auditory pathway has not been clarified yet. The second purpose of the present study was, therefore, to identify molecular markers to understand the molecular mechanism of salicylate-induced tinnitus in the rat auditory pathway. Salicylate inhibits cyclo-oxygenase activity (Christie et al., 1998) and cyclo-oxygenase inhibition leads to the *in vitro* activation of a nociceptive receptor transient receptor potential cation channel superfamily V-1 (TRPV1) (Fosslien, 1998; Hwang et al., 2000; Caterina et al., 1997; Benham et al., 2003). TRPV1 is located in the mouse inner ear ganglia and is upregulated *in vivo* after noxious challenges of kanamycin (Kitahara et al., 2005a). Furthermore, cochlear background activity is increased by inner ear perfusion of capsaicin, a TRPV1 agonist and is suppressed by capsazepine, a TRPV1 specific antagonist (Zhou et al., 2006). We examined changes in mRNA and protein levels of TRPV1 in the salicylate-treated rat auditory pathway. Although salicylate-induced tinnitus is acute and transient, we believe that by elucidating the molecular mechanism of salicylate-induced tinnitus we can provide insight into chronic intractable tinnitus.

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Abbreviations: ABR, auditory brainstem response; BRCx, brain cortex; B2m, beta-2 microglobulin; CT, cycle threshold; DCN, dorsal cochlear nucleus; DMSO, dimethyl sulfoxide; RT, room temperature; SG, spiral ganglion; SPL, sound pressure level; TRPV1, transient receptor potential cation channel superfamily V-1.

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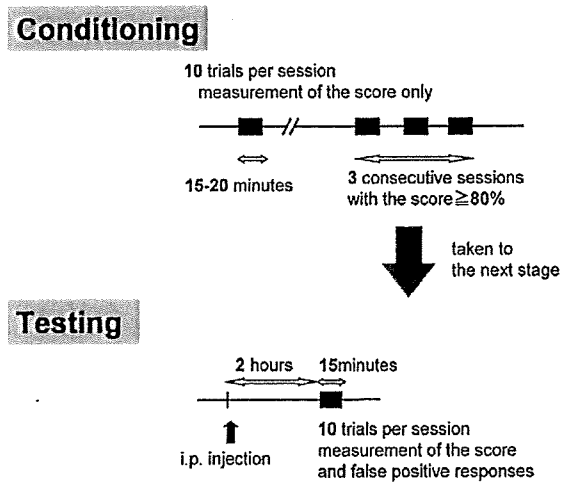

Fig. 1. Schematic representation of the behavioral protocol. In "conditioning," animals were conditioned to move in response to a sound stimulation. The conditioning procedure requires up to seven sessions lasting 15–20 min. When conditioned (criterion, three consecutive sessions with an active avoidance score ≥80%), animals were taken to the next stage, "testing" (day 0). The "testing" behavioral protocol consisted of a daily measurement (on four consecutive days, days 1–4) of the correct responses to sound (active avoidance score) and moves during inter-trial periods (false positive responses) in a 15 min session. Saline or salicylate was injected daily 2 h before the testing session.

EXPERIMENTAL PROCEDURES

Experimental procedures involving animals were performed according to animal ethical guidelines and were approved by Osaka University, School of Medicine (certificate number: 0755). All efforts were made to minimize the suffering to animals and to limit the number of animals used. A total of 99 male Wistar rats (Japan SLC, Hamamatsu, Shizuoka, Japan), weighting between 150 and 200 g, were used. Animals were individually housed in a temperature-controlled room on a constant 12 h light/dark cycle. All behavioral tests were conducted during the animals' activity period (dark phase) at approximately the same time each day. Food and tap water were available throughout the experiments.

Behavioral assessment

Animals were trained to perform "an active avoidance task," according to the protocol of Guitton et al. (2003) (Fig. 1). Both "conditioning" and "testing" were performed in a conditioning box that had an electrified floor, which was divided in two by a low wall, 3 cm high. The conditioning box was in a soundproof room. A 5 s pure tone sound was used as the "conditioned stimulus" and a 3.7 mA electrical footshock was given for a maximum of 30 s as the "unconditioned stimulus." The interval between conditioned and unconditioned stimuli was 1 s. The footshock was stopped when animals correctly escaped from the unconditioned shock to the opposite side of the cage. The inter-trial interval or silent period was at least 1 min. The level of performance over 10 trials, or "active avoidance score," was assessed by the ratio of how many times the rat moved correctly in response to the conditioned sound. In the "conditioning" stage, animals were considered to be conditioned when the active avoidance score reached at least 80% in three consecutive sessions. When conditioned, animals were taken to the next "testing" stage (day 0).

Testing was performed once daily for 4 days, at the same time each day (day 1–4). Animals received a single daily injection of saline or sodium salicylate (400 mg/kg i.p.) (Sigma, St. Louis, MO, USA) for 3 days (day 1–3), according to the previous reports of Jastreboff and Sasaki (1986); Rüttiger et al. (2003) and Im et al. (2007). Injections were performed 2 h before behavioral measurements. On the fourth day, they received no treatment. The behavioral protocol consisted of a daily measurement of the active avoidance score and of false positive responses. The "false positive responses" represent the number of movements to the opposite side of the cage during the inter-trial interval, when there was no sound. Trials were randomized and electric footshocks were presented only if animals didn't move in response to sounds. Whatever the results of the active avoidance score and false positive responses were, each session included 10 trials and lasted 15 min. Both the active avoidance score and false positive responses were measured in the same session.

The most appropriate conditioned stimulus was determined by the following pilot experiments. Animals were divided into four groups (n=6 in each group) according to conditioned stimuli of 4, 10, 16 or 40 kHz (60 dB sound pressure level (SPL)), and false positive responses were measured on the third day after salicylate injection. Animals were also divided into three groups (n=6 in each group) according to conditioned stimuli of 20, 60 or 80 dB SPL (16 kHz) and false positive responses were measured as above.

Auditory brainstem response (ABR) recording

The ABR was measured with a Neuropack-4 (Nihon Koden Co., Shinjuku, Tokyo, Japan). The active platinum electrode was inserted at the vertex, and reference electrodes at both pinnae of the ears. Binaural, open fielded stimuli of click were generated through a TDH-49 headphone attached to the animal's ears. The rat ABR consisted of a series of III to V vertex-positive peaks within the first 6 ms from the onset of the stimulus, and they were called I to V. The III wave was detected at the lowest stimulus intensity, so the threshold was defined as the lowest stimulus intensity to elicit a reliable III wave.

Hematoxylin and eosin staining

Serial inner ear sections from saline controls and salicylate-treated rats were stained with hematoxylin and eosin to determine if salicylate treatment caused any overt damage to the inner ear. Morphological structures in the organ of Corti and the spiral ganglion (SG) were microscopically observed.

Analysis of mRNA levels

Animals were divided into five groups: a saline i.p. injection control group, a 2 h post-salicylate i.p. injection group, a 12 h post-salicylate injection group, a 24 h post-salicylate injection group, and a 72 h post-salicylate injection group (n=6 in each group).

The procedures of tissue preparation for real-time PCR have already been described in our previous papers (Kitahara et al., 2005a,b). Animals were deeply anesthetized with pentobarbital and the SG, dorsal cochlear nucleus (DCN) and brain cortex (BRCx) were immediately dissected under a stereomicroscope in chilled buffered saline and then frozen in dry ice powder. The DCN region is thought to be one of the main structures involved in tinnitus (Eggermont and Roberts, 2004) and it was carefully dissected according to the coordinates of the Paxinos and Watson brain atlas; rostral coordinate, bregma: −10.52 mm, and caudal coordinate, bregma: −11.60 mm (Paxinos and Watson, 1986). The BRCx region does not include the auditory cortex. Total RNA was extracted using an RNeasy Mini Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions.

PCR was performed using oligonucleotide primers for TRPV1 (Takara Bio Inc., Otsu, Shiga, Japan) and beta-2 microglobulin (B2m) (Takara), as shown in Table 1, and products were quantified by SYBR Green PCR reagents (Applied Biosystems, Foster City, CA, USA). B2m was assayed as a control housekeeping gene. The PCR mixture included 10 μl of 2× SYBR Premix Taq,

Table 1. Gene-specific primers for PCR of rat transient receptor potential cation channel superfamily V type 1 (TRPV1) and β-2 microglobulin (B2m)

TRPV1 (accession no. NM_031982)
Forward primer 5′-ACTCCTGACGGCAAGGATGAC-3′
Reverse primer 5′-ACCCACATTGGTGTTCCAGGTAG-3′
Estimated size 81 bp
B2m (accession no. NM_012512)
Forward primer 5′-CCTGGCTCACACACTGAATTCACAC-3′
Reverse primer 5′-AACCGGATCTGGAGTTAAACTGGTC-3′
Estimated size 163 bp

0.8 μl of each gene-specific primer (5 μM), 6.8 μl of dH2O, 0.4 μl of 50× ROX Reference Dye and 2 μl of cDNA (250 ng) in a final volume of 20 μl. The conditions used were 95 °C for 10 s, 40 cycles at 95 °C for 5 s and 60 °C for 30 s, 95 °C for 15 s, 60 °C for 1 min and 95 °C for 15 s. The amplification plots from fluorescent emission data collected during PCR were constructed using the ABI7900 model software (Applied Biosystems).

The number of PCR cycles was recorded until the fluorescence intensity exceeded the pre-determined threshold. The quantification of the initial amounts of template molecules relied on this number of PCR cycles, which is termed the cycle threshold (CT). The dCT represents the CT of the target gene normalized to the rat endogenous B2m ($dCT=CT_{target}-CT_{B2m}$). Relative quantification of the mRNA levels of target genes (=fold range) was calculated using the 2^{-ddCT} method, where $ddCT=(CT_{target}-CT_{B2m})A-(CT_{target}-CT_{B2m})B$ (Schmittgen et al., 2000). For example, changes in gene expression of TRPV1 among groups CONT, SA2H, SA12H, SA24H and SA72H ($GROUP_x$) within each region were quantified as the fold range: 2^{-ddCT} ($ddCT=(CT_{TRPV1}-CT_{B2m})_{GROUPx}-(CT_{TRPV1}-CT_{B2m})_{CONT}$).

Protein examination

TRPV1 protein levels were assayed using standard western blotting techniques (Kitahara et al., 2005a,b). Briefly, tissues of each region (SG, DCN, BRCx) were obtained from a saline i.p. injection control group, a 2 h post-salicylate i.p. injection group and a 24 h post-salicylate injection group (n=8 in each group) through the same procedure for real-time PCR in the present study. Then, the tissues were added to lysis buffer (1% NP-40, 150 mM NaCl, 1 mM EDTA, 10 mM PBS (pH 7.4), 0.25 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin) and homogenized gently on ice using a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY, USA). The samples were boiled for 1 min, transferred to clean Eppendorf tubes on ice and centrifuged at 10,000 g for 30 min. Supernatant was transferred to clean tubes, and protein concentration was determined using a protein assay kit (Pierce, Rockford, IL, USA). The protein extracts (20 μg for each lane) were subjected to 12% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose filters (Amersham Biosciences, Piscataway, NJ, USA) at 500 mA for 1 h. The filters were pre-blocked in 0.1 M PBS containing 0.2% Tween 20 and 5% non-fat dried milk for 3 h at room temperature (RT), probed overnight with TRPV1 primary antibody (anti-rabbit polyclonal; diluted 1:1000; Alpha Diagnostic International, San Antonio, TX, USA) and then washed with Blotto solution (50 mM Tris (pH 7.4), 0.9% NaCl, 0.5% Tween 20) for 1 h. Filters were then incubated with HRP-conjugated secondary antibody (Dako, Carpenteria, CA, USA) for 1 h and washed with Blotto solution for 1 h. Protein bands were visualized using Supersignal Ultra chemiluminescence substrate (Pierce), assessed on ECL film (Amersham Biosciences) and quantified by laser densitometry (Quantity One Software, Bio-Rad Lab, Hercules, CA, USA). The density of staining with anti-β actin monoclonal antibody (Oncogene Research Products, Boston, MA, USA; diluted 1:500) was used to normalize the TRPV1 densitometric determination of each sample.

Tissue preparation procedures for immunohistochemistry have already been described in our previous papers (Kitahara et al., 2005a,b). Temporal bones were obtained from two adult male rats from each of a saline i.p. injection control group and a 2 h post-salicylate i.p. injection group. The animals were euthanized with sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially with 0.1 M PBS, followed by paraformaldehyde-lysine-periodate fixative. Temporal bones were post-fixed in 4% paraformaldehyde for 24 h at RT, decalcified in 10% formic acid, neutralized overnight in 5% sodium sulfate, infiltrated with OCT compound and sectioned on a cryostat at 5 μm in a plane that produces mid-modiolar sections. The sections were thaw mounted on slides. The 5 μm cryostat sections were incubated sequentially in the following solutions at RT: 0.1% TritonX-100 and 2% bovine serum albumin (BSA) in 0.1 M PBS for 2 h; antisera against TRPV1 (anti-rabbit polyclonal; diluted 1:1000; Alpha Diagnostic International) in the above solution for 48 h; 0.1 M PBS for 15 min; biotinylated goat anti-rabbit IgG (diluted 1:250; Vector Laboratories, Burlingame, CA, USA) in 2% BSA in 0.1 M PBS for 24 h; 0.1 M PBS for 15 min; Vectastain ABC reagent (Vector Laboratories) for 1 h; 0.1 M PBS for 15 min; 5 mg/ml diaminobenzidine tetrahydrochloride (DAB)/0.01% H_2O_2 in 0.05 M Tris buffer for 5 min. Sections were then examined under a light microscope. For negative controls, primary antibodies were either pre-absorbed with each control peptide (1:50) or the primary antibody was omitted.

Antagonist treatment

To elucidate the direct involvement of TRPV1 in the mechanism of tinnitus generation, a specific antagonist of TRPV1, capsazepine (Sigma) and its vehicle, 50% dimetyl sulfoxide (DMSO) (Sigma) were used. Group I was a saline+DMSO group, group II was a salicylate+DMSO group, group III was a salicylate+capsazepine group and group IV was a saline+capsazepine group. DMSO or capsazepine was administrated 0.5 h after the first injection of saline or salicylate. Effects of capsazepine within the dose range of 0–10 mg/kg (De Schepper et al., 2008) were evaluated behaviorally by counting false positive responses (cf. 2.1 in Experimental Procedures) and morphologically by real-time PCR (cf. 2.3 in Experimental Procedures).

Statistical analysis

In the present paper, the statistical significance of changes among groups was analyzed using one-way ANOVA with Bonferroni t-test except for capsazepine treatment. Two-way ANOVA with Bonferroni t-test was used to test the hypotheses that salicylate effect is blocked by capsazepine. All the data were presented as mean±SE and P-values under 0.05 were considered significant (SPSS Inc., Chicago, IL, USA).

RESULTS

Behavioral assessment

In pilot experiments, animals were divided into four groups (n=6 in each group) according to conditioned stimuli of 4, 10, 16 or 40 kHz (60 dB SPL), and false positive responses were measured on the third day after salicylate injection. Each number of false positive responses was 0.6±0.4 (4 kHz), 2.8±0.8 (10 kHz), 4.2±0.9 (16 kHz), or 0.6±0.4 (40 kHz) and increased significantly when the conditioned stimulus was 16 kHz (* P=0.004) (Fig. 2A). Then, animals were also divided into three groups (n=6 in each group) according to conditioned stimuli of 20, 60 or 80 dB SPL (16

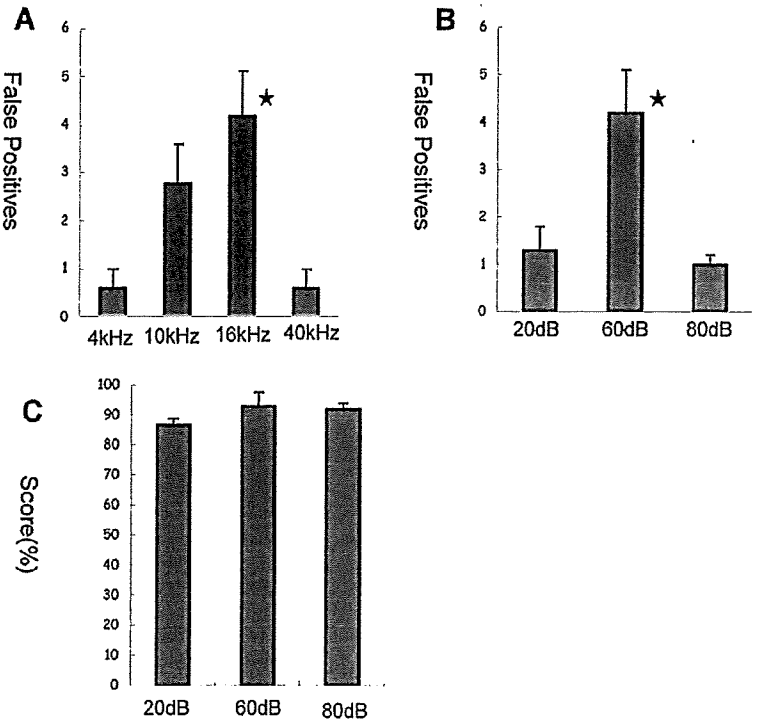

Fig. 2. The most appropriate kHz and dB sound pressure level (SPL) for the conditioning stimulus in the rat salicylate-induced tinnitus model. (A) Animals were divided into four groups (n=6 in each group) according to conditioning stimuli of 4, 10, 16 or 40 kHz (60 dB SPL). The number of false positives were measured on the third day after salicylate injection and increased significantly when the conditioning stimulus was 16 kHz (* P<0.005). (B) Animals were divided into three groups (n=6 in each group) according to conditioning stimuli of 20, 60 or 80 dB SPL (16 kHz). The number of false positives between these groups on the third day after salicylate injection increased significantly when the conditioning stimulus was at 60 dB SPL (* P<0.05). (C) The percentage of correct responses (score %) to sound of 20, 60 or 80 dB SPL (16 kHz) (active avoidance score). There were no significant differences between all three groups.

kHz) and false positive responses were measured as above. Each number of false positive responses was 1.3±0.5 (20 dB SPL), 4.2±0.9 (60 dB SPL), or 1.0±0.2 (80 dB SPL) and increased significantly when the conditioned stimulus was 60 dB SPL (* P=0.013) (Fig. 2B). The active avoidance score showed no significant change among all these groups, though (Fig. 2C). From these experiments, a 16 kHz and 60 dB SPL pure tone sound was adopted as the most appropriate conditioned stimulus for the present Wistar rat study.

Animals in the control group showed no significant change either in the active avoidance score (Fig. 3A) or in the false positive responses (Fig. 3B) from day 0 to day 4. The active avoidance scores of control and experimental

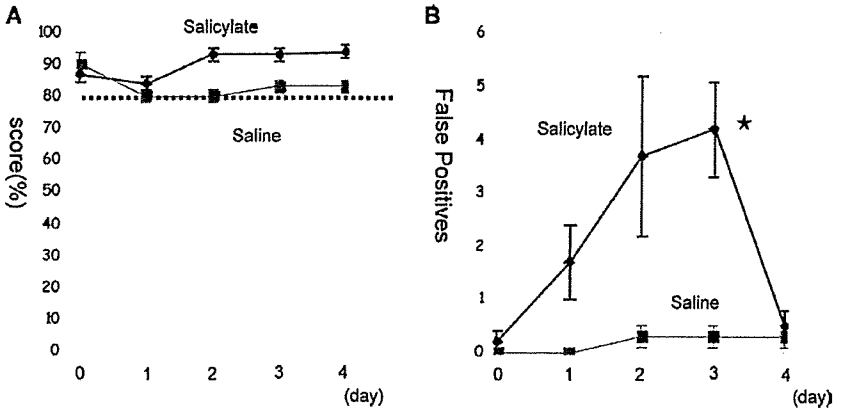

Fig. 3. The active avoidance score (A) and false positive responses (B) in salicylate-treated rats. (A) The percentage of correct responses to sound (active avoidance score %) measured before (day 0), during (day 1–3), and after injections of saline or salicylate (400 mg/kg i.p.) (day 4). The active avoidance score % remained stable (≥80%) in both groups during the experimental period. (B) The number of abnormal responses during silent periods (false positives). Injections of salicylate significantly increased the number of false positives on the third day (day 3) (* P<0.005). A complete recovery was seen when the treatment was stopped on the fourth day (day 4).

animals remained stable (≥80%) during the whole period of saline and salicylate administration. This suggests that salicylate application did not cause any obvious functional damage to cochlear endo-organs. False positive responses increased gradually after salicylate treatment with a maximum at day 3 (day 1: 0.2±0.2, day 2: 1.7±0.7, day 3: 4.2±0.9, day 4: 0.5±0.3, * P=0.002) and returned to the control value at day 4. This suggests that salicylate treatment caused a phantom sound sensation or "tinnitus" in rats, with a maximum at day 3 that endured for one day at most.

ABR assessment

We examined if the salicylate induced the inner ear dysfunction by means of ABR. We used the III wave in the measurement of ABR threshold, because this was most detectable at lower-intensity stimuli reference. No remarkable differences were observed between mean ABR threshold of control and salicylate-treated rats (data not shown). This suggests that salicylate treatment did not cause any obvious functional damage to cochlear endo-organs.

Morphological assessment

Neither hair cells nor SG cells showed any remarkable changes between control and salicylate-treated rats (data not shown). This suggests that salicylate treatment did not cause any obvious morphological damage to cochlear endo-organs.

Expression of TRPV1 in the auditory pathway and BRCx

TRPV1 mRNA levels in the SG were significantly upregulated 2 h (2.39±0.18 fold: * P=0.0005), returned to control levels 12 h (0.81±0.02 fold: P=0.105), significantly suppressed 24 h (0.35±0.03 fold: ** P=0.0002) and returned to control levels 72 h post-treatment (0.91±0.05 fold: P=0.130) (Fig. 4A). TRPV1 mRNA levels in the DCN were significantly suppressed 2 h (0.53±0.03 fold: * P=0.0001) and 12 h (0.64±0.03 fold: * P=0.0001) post-treatment, respectively and returned to control levels 24 h post-treatment (1.01±0.05 fold: P=0.810) (Fig. 4B). TRPV1 mRNA levels in the BRCx did not show any significant change after salicylate treatment (Fig. 4C).

TRPV1 protein levels in the inner ear

Fig. 5 illustrates western blot results for TRPV1 and the relative protein levels (TRPV1/actin in CONT=1) in the rat SG, DCN and BRCx. Similar to the results for mRNA levels, TRPV1 protein levels in the SG were significantly increased 2 h after salicylate treatment (2.94±0.72: * P=0.022) and returned to control levels 24 h after salicylate treatment (0.89±0.65: P=0.068) (Fig. 5A). In spite of a slight reduction 2 h after salicylate treatment (0.61±0.48: * P=0.044), TRPV1 protein levels in the DCN were significantly increased 24 h after salicylate treatment (1.56±0.60: ** P=0.038) (Fig. 5C). TRPV1 protein levels in the BRCx did not show any significant change after salicylate treatment (Fig. 5D).

TRPV1 immunoreactivity in the salicylate-treated group was clearly enhanced in almost all SG cells compared with that in the saline-control group (Fig. 5B). The dense immunoreactivity extended beyond the perinuclear region and into the somata of ganglion cells. All TRPV1 immunoreactivity was eliminated by pre-absorption with an excess of blocking peptide (data not shown).

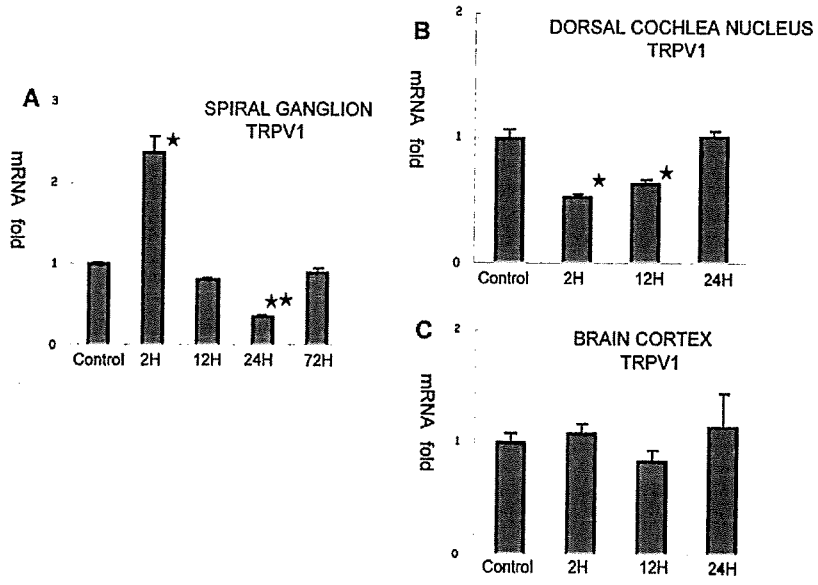

Fig. 4. Salicylate-induced changes in transient receptor potential cation channel superfamily V-1 (TRPV1) mRNA levels in the rat spiral ganglion (SG) (A), dorsal cochlear nucleus (DCN) (B) and brain cortex (BRCx) (C). (A) TRPV1 mRNA levels in SG were significantly upregulated 2 h (* P<0.001), returned to control levels 12 h, significantly suppressed 24 h (** P<0.0005) and returned to control levels 72 h post-treatment. (B) TRPV1 mRNA levels in the DCN were significantly suppressed at the 2 and 12 h post-treatment (* P<0.0005) and had returned to control levels 24 h post-treatment. (C) TRPV1 mRNA levels in the BRCx did not show any significant change after salicylate treatment.

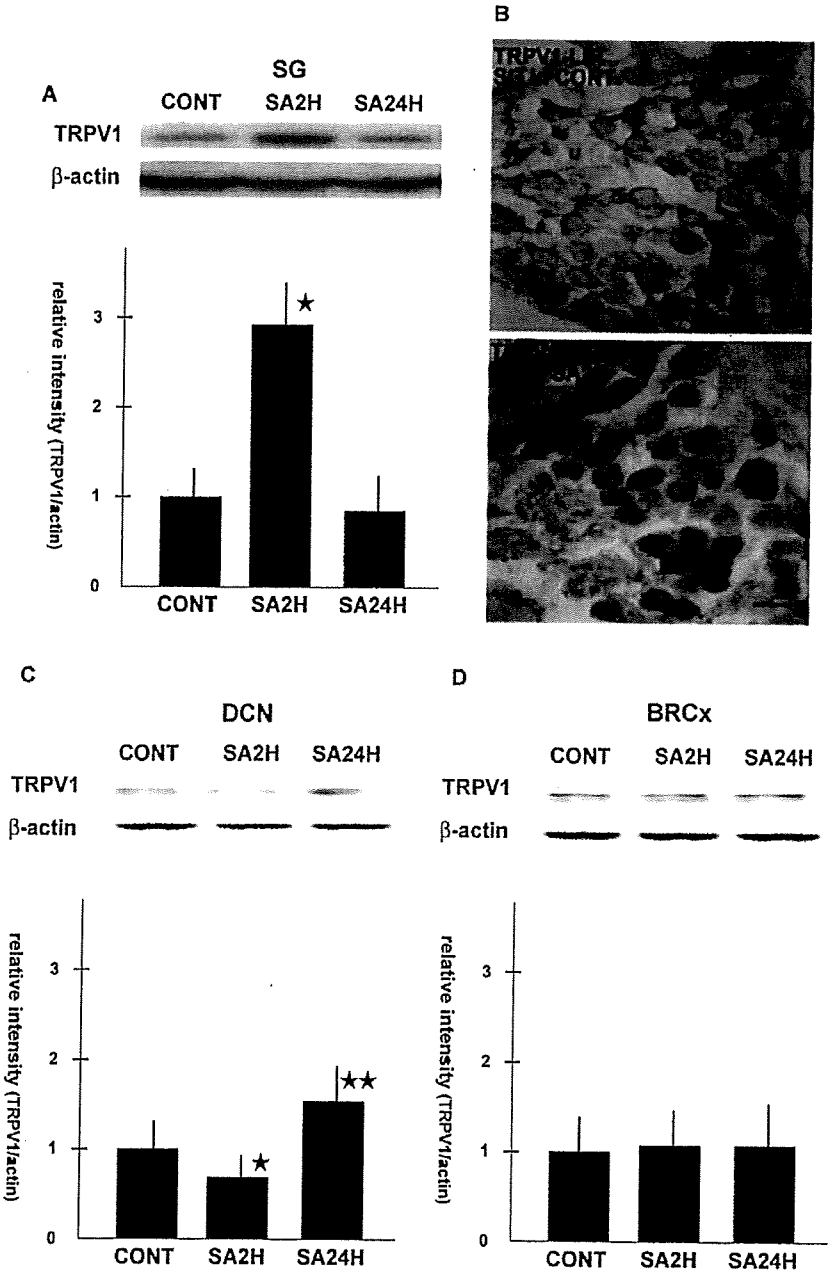

Fig. 5. Western blot analysis (A, C, D) and immunohistochemistry (B) of salicylate-induced changes in TRPV1 protein levels in the rat SG, DCN and BRCx. (A) TRPV1 protein levels in SG were significantly increased 2 h after salicylate treatment (SA2H) (* $P<0.05$) and had returned to control levels (CONT) 24 h after salicylate treatment (SA24H). (B) TRPV1-like immunoreactivity (LIR) in the salicylate-treated group (SA2H) was clearly enhanced in almost all SG cells compared with that in the saline-control group (CONT). (C) In spite of a slight reduction 2 h after salicylate treatment (SA2H) (* $P<0.05$), TRPV1 protein levels in the DCN were significantly increased 24 h after salicylate treatment (SA24H) (** $P<0.05$). (D) TRPV1 protein levels in the BRCx did not show any significant change after salicylate treatment.

Effect of TRPV1 antagonist

Behavioral assessment showed that false positive responses were increased 2 h post-treatment in group II (salicylate+DMSO) (3.7±1.2 responses) compared with control group I and that this increase was significantly suppressed in group III (salicylate+capsazepine) (0.8±0.5 responses, * $P=0.015$) (Fig. 6A). Examination of TRPV1 mRNA levels in the SG showed that mRNA was upregulated 2 h post-treatment in group II (salicylate+DMSO) (3.38±1.13 fold) compared with the control group I and that this upregulation was significantly suppressed in group III (salicylate+capsazepine) (0.85±0.07 fold, * $P=0.020$) (Fig. 6B). Capsazepine suppressed the salicylate-induced molecular changes in the SG in a dose dependent manner (Fig. 6C).

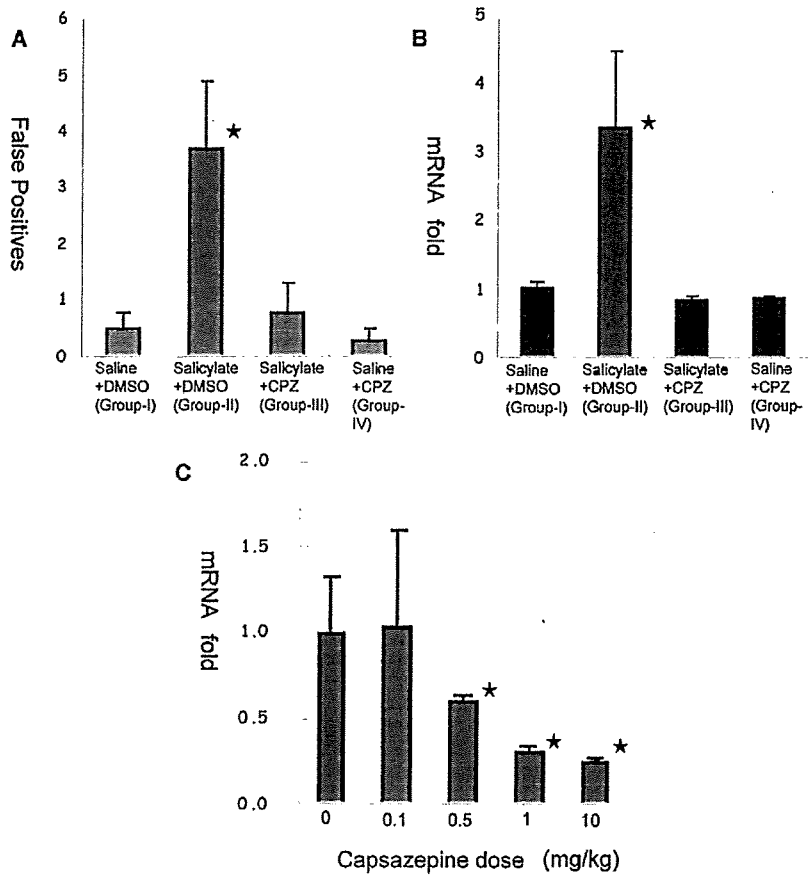

Fig. 6. Capsazepine (CPZ) blocked both the salicylate-induced increase of false positives and upregulation of TRPV1 mRNA in the rat SG. Dimethyl sulfoxide (DMSO) or CPZ (10 mg/kg i.p.) was administrated 0.5 h after the first injection of saline or salicylate. (A) False positives were increased 2 h post-treatment in group II (salicylate+DMSO) and this increase was significantly suppressed in group III (salicylate+CPZ) (* $P<0.05$). (B) TRPV1 mRNA was upregulated in the SG 2 h post-treatment in group II (salicylate+DMSO) and this upregulation was significantly suppressed in group III (salicylate+CPZ) (* $P<0.05$). (C) CPZ (0–10 mg/kg) suppressed the salicylate-induced upregulation of TRPV1 mRNA in the SG in a dose dependent manner (* $P<0.05$).

DISCUSSION

One of the reasons why it is still so hard for clinicians to cure intractable tinnitus is that appropriate animal models of tinnitus have not yet been established. To overcome this deficiency, we present here an animal behavioral model of tinnitus. Validating a behavioral procedure in animals to assess the presence of tinnitus is an unusual and difficult task to which many research groups have devoted many years. Among these groups, Jastreboff and Sasaki (1994) proposed an animal model based on an active avoidance paradigm; animals were conditioned by electrical footshock to drink water when hearing a sound. Recently, Guitton et al. (2003) demonstrated an animal model involving escape to a climbing pole to avoid electrical footshock when hearing a sound. In the present study, we further developed Guitton's model with modifications of a much easier active avoidance task of escaping to the next room instead. Adult or even aged animals were able to quickly step over the wall to move to the other side of the box. The conditioning box should also be set in a soundproof room, because it was important for animals to clearly hear both the conditioned sound and salicylate-induced phantom sound.

Previous studies in tinnitus kHz have revealed that salicylate induces an acute, relatively high frequency and transient type of tinnitus. It ranges from 10 to 16 kHz (Bauer et al., 1999; Guitton et al., 2003; Zheng et al., 2006; Yang et al., 2007). In the present study, false positive responses increased significantly when the conditioned stimulus was 16 kHz compared with 4, 10 and 40 kHz. Previous studies in tinnitus dB SPL have also shown that salicylate caused tinnitus of around 60 dB SPL (Bauer et al., 1999; Guitton et al., 2003; Rüttiger et al., 2003; Zheng et al., 2006; Yang et al., 2007). In the present study, false positive responses also increased significantly when the conditioned stimulus was 60 dB SPL compared with 20 dB SPL and 80 dB SPL. Judging from these pilot experiments, together with previous papers, 16 kHz and 60 dB SPL pure tone sound was the most similar to the sound of 400 mg/kg salicylate-induced tinnitus in the present Wistar rat study.

Another reason why it is still so hard for clinicians to cure intractable tinnitus is that the molecular mechanism of tinnitus generation in the auditory pathway has not yet been clarified. We therefore aimed to establish such a molecular marker. The history of TRP channels in hearing and balance is characterized at great length by the hunt for the elusive transduction channel of sensory hair cells. Such pursuit has not resulted in unequivocal identification of the transduction channel, but nevertheless revealed a number of candidates, such as TRPV4, TRPN1, TRPA1, and TRPML3. Based on mutations in the corresponding mouse genes, TRPV4 (Tabuchi et al., 2005; Cuajungco et al., 2007) and TRPML3 (van Aken et al., 2008) are possible candidates for human hearing, and potentially also balance disorders. In the present study, we focused especially on TRPV1, a member of the non-specific cation ion channel receptor family, which responds to various kinds of noxious pain, such as capsaicin, inflammation, heat, low pH and hypo-osmolarity (Caterina et al., 1997; Benham et al., 2003), because it is expressed in the mouse inner ear ganglia and is upregulated by noxious challenges of kanamycin (Kitahara et al., 2005a). Interestingly, tinnitus is the sensation of a sound in the ear without an external source, similar to phantom pain (Bartels et al., 2007).

In the present study, TRPV1 mRNA levels in the SG were significantly upregulated 2 h post-treatment, significantly downregulated 12–24 h post-treatment and had returned to control levels by 72 h post-treatment. The reasons of discrepancy in these molecular results of mRNA and protein level could be explained by the time lag between mRNA and protein synthesis and/or different sensitivity in mRNA and protein experiments. According to the animal behavioral model of tinnitus, salicylate-induced tinnitus was maximal 2 h post-injection and had disappeared by 24 h post-injection. Furthermore, capsazepine, a TRPV1 antagonist, demonstrated a significant suppression of false positive response increase and of TRPV1 mRNA upregulation in the SG. Taken together, these data suggest that tinnitus in salicylate-treated animals could be caused through the activation of the nociceptive receptor, TRPV1 in the SG. Therefore, we hypothesize that tinnitus is a type of phantom pain sensation in the inner ear (Bartels et al., 2007). The mechanism of TRPV1 regulation in the SG has not been clarified yet. However, TRPV1 is auto-regulated via neurotrophic factors in damaged dorsal root ganglia (Acheson et al., 1995; Anand et al., 2006; Szallasi et al., 2006). In the present study, the blockade of salicylate-induced TRPV1 upregulation in the SG by capsazepine suggests that TRPV1 was also auto-regulated via neurotrophic factors in the salicylate-treated SG (Hansen et al., 2001; Zha et al., 2001; Shepherd et al., 2005; Kitahara et al., 2006). Known TRPV1 antagonists (capsazepine, BCTC and thio-BCTC) were also able to block the response of TRPM8 (Behrendt et al., 2004), which shares many functional and pharmacological properties with TRPV1 (Weil et al., 2005). Although TRPM8 has never been reported to be located in the inner ear, the possible role of TRPM8 in hearing and/or tinnitus should be discussed after further studies of TRPM8 in the inner ear.

The following mechanisms of salicylate-induced TRPV1 activation and tinnitus generation are suggested: TRPV1 and 5-lipoxygenase are co-expressed by SG cells in the inner ear (Balaban et al., 2003). Salicylate, an active component of aspirin, inhibits cyclo-oxygenase activity (Christie et al., 1998) and this cyclo-oxygenase inhibition leads to an excess of intracellular arachidonic acid, which is metabolized by 5-lipoxygenase pathways (Fosslien, 1998). These findings suggest that the resultant increase in arachidonic acid products, such as hydroperoxyeicosatetraenoic acid and hydroxyeicosatetraenoic acid, has the potential to depolarize SG cells by activation of TRPV1 (Hwang et al., 2000). This may either lower their threshold for spike generation or increase their sensitivity to suprathreshold activation and mimic the discharge pattern during low level natural stimulation. Actually, a couple of physiological studies of TRPV1 in the cochlea were reported. Zheng et al. revealed that activation of TRPV1 increases the threshold of the cochlear action potential, but decreases both cochlear microphonic and electrically-evoked otoacoustic emissions (Zheng et al., 2003). Zhou et al. demonstrated that perfusion with capsaicin alone produced a dose-dependent increase of the 900 Hz peak ratio (power normalized re the overall spectrum) of the ensemble background activity (Zhou et al., 2006). The capsaicin effect was attenuated during concurrent perfusion with capsazepine. These findings are consistent with the hypothesis that TRPV1 activation increases background activity of SG cells and support a role of TRPV1 in gating spontaneous and evoked auditory nerve excitability.

In contrast, the peak TRPV1 protein levels in the DCN were delayed relative to the peak levels in SG cells. This delay might indicate the mechanism for the alteration from peripheral tinnitus to central tinnitus and/or the mechanism of chronic tinnitus. There is an interesting case of a patient with intractable chronic tinnitus, who underwent temporal bone removal surgery, however, this treatment failed to cure the tinnitus (House, 1964). It is extremely difficult to identify the site of chronic tinnitus, because it may alternate between the periphery and the CNS, like phantom pain sensation. Further studies addressing the mechanisms of central tinnitus and/or chronic tinnitus are needed.

According to the morphological data, salicylate treatment caused no obvious morphological damage to cochlear hair cells or SG cells. Furthermore, from the behavioral study, the active avoidance score remained stable during the whole period of salicylate injections. Together with the ABR data, this suggests that salicylate application caused no obvious functional damage to the auditory system. However, Guitton et al. (2005) reported that administering salicylate led to transient hearing loss by means of compound action potential (CAP) threshold shifts, directional preponderance of otoacoustic emission (DPOAE) recordings and score measurements. This hearing loss was evaluated around 40 dB SPL at 16 kHz (Cazals, 2000; Guitton et al., 2005) and might not be comparable to the results in the present study, which used 60 dB SPL and 16 kHz sound stimuli.

CONCLUSION

In conclusion, we developed a rat behavioral model of salicylate-induced tinnitus and identified a molecular marker of salicylate-induced tinnitus in the rat auditory pathway. These findings could make "phantom tinnitus" clearly observable and easily accessible. We believe that these findings are important for understanding the mechanism of tinnitus generation and for elucidating de novo treatments for intractable tinnitus.

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JOHNS
特集●耳の奇形●

症状・診断

内耳奇形の聴覚検査所見

熊川孝三*
Kozo KUMAKAWA

● Key Words ●内耳奇形，前庭水管拡大症，内耳道狭窄症●

はじめに

骨迷路の形態異常を伴う内耳奇形の頻度は一般に考えられているよりも高く，Jensen[1]は先天性高度感音難聴者の20％に認められると報告している。その分類には多くのものがある。

病理組織学的には，

1）Michel型：内耳の完全欠損
2）Mondini型：骨迷路，膜迷路が不完全に発育
3）Bing-Siebenmann型：骨迷路は正常で膜迷路，感覚細胞が不完全に発育
4）Scheibe型：蝸牛の膜迷路と球形嚢に障害があるが前庭膜迷路と骨迷路は正常に発育

などと分類されることが多い。しかし，このような分類は病理組織学的検査によって初めて可能になるもので，実際の画像・臨床診断には適さない。

そこでJacklerら[2]はこれらの点を考慮して表1のような分類を提唱している。これは，まず，膜迷路に限局した奇形と，骨迷路と膜迷路の異常を合併するものに大きく分け，さらに後者を，

A：内耳の無形成
B：蝸牛奇形を伴うもの
C：蝸牛の異常を伴わず半規管・前庭の迷路奇形のみを伴うもの
D：水管の奇形
E：内耳道の奇形

とに分ける分類である。これらの分類は画像診断の所見に基づいており，臨床診断に適している。ここではこの分類にしたがって内耳奇形を分類し，それぞれの聴覚検査所見について述べる。

表1　内耳奇形の分類（Jacklerら）[2]

Ⅰ．膜迷路に限局した奇形
　A．膜迷路の完全異形成
　B．膜迷路の限局した異形成
　　1）蝸牛，球形嚢の異形成（Scheibe）
　　2）蝸牛基底回転の異形成（Alexander）
Ⅱ．骨迷路と膜迷路の奇形
　A．迷路の無形成（Michel）
　B．蝸牛の奇形
　　1）蝸牛の無形成
　　2）蝸牛の低形成
　　3）骨隔壁の低形成（Mondini）
　　4）共通腔（common cavity）
　C．迷路奇形
　　1）半規管異形成
　　2）半規管無形成
　D．水管の奇形
　　1）前庭水管拡大
　　2）蝸牛水管拡大
　E．内耳道の奇形
　　1）内耳道狭窄
　　2）内耳道拡大

Ⅰ．内耳奇形の型と聴力像

Jacklerらの分類[2]にしたがって，彼らの聴力レベルの解析結果を表2に示す。図1は蝸牛の異常を伴う内耳奇形について，図2は蝸牛の異常を伴わない半規管・前庭の内耳奇形について，筆者[3]が改変し，それぞれ発達が停止した胎生時期順に示した。

Michel型は内耳の完全欠損であり，当然ながら聴力はスケールアウトである。

Bの蝸牛奇形を伴う群の中で最も頻度が高いものは，一般的にMondini型奇形と呼ばれている奇

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表 2　内耳奇形の分類と平均聴力（Jackler ら）[2]

型	耳数	0～40	41～80	81～100	101～120	>120 dB *
A 内耳の完全欠損（Michel）	1	—	—	—	—	1
B 蝸牛奇形を伴うもの						
蝸牛・前庭が共通腔	19	1	6	—	3	9
蝸牛無形成	2	—	—	—	1	1
蝸牛低形成	11	6	3	1	—	1
蝸牛回転の隔壁欠損（Mondini）	41	7	15	4	7	8
C 蝸牛奇形を伴わないもの						
前庭・半規管の形成不全	7	5	—	1	1	—
D 前庭水管の拡大	17	7	4	4	2	—

＊ 500，1000，2000 Hz の平均聴力

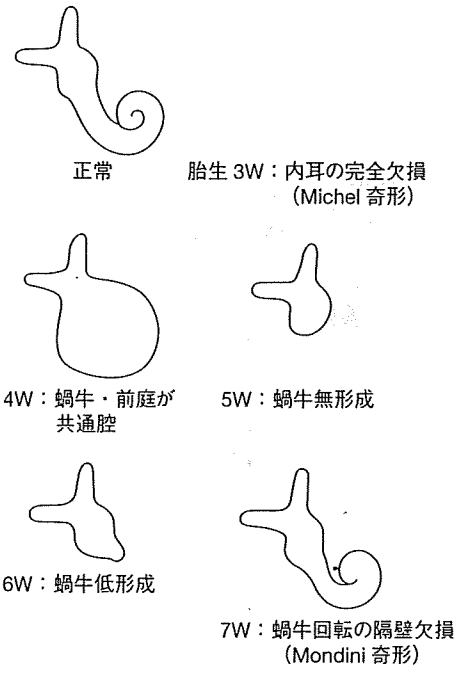

図 1　蝸牛の異常を伴う内耳奇形（Jackler[2] より改変）[3]
発達が停止した胎生時期を示す。

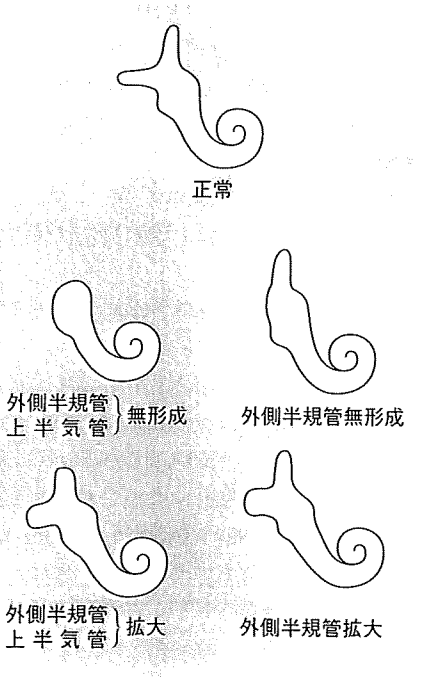

図 2　蝸牛の異常を伴わない内耳奇形（Jackler[2] より改変）[3]
胎生 6 週～22 週における発達の停止による。

形である。これは，胎生 7 週目の異常で，蝸牛軸が不完全で基底回転（第 1 回転）までは発達しているが，それよりも distal の回転間の隔壁が欠損し，中・頂回転が合わさって嚢状に置き換わったものである。コルチ器・有毛細胞と聴神経終末の欠損程度にはバラツキがあり，表 2 に示したように難聴も軽度から高度までさまざまである。

次に多いのは胎生 4 週目の異常で，蝸牛と前庭が大きな共通した腔となって蝸牛の回転が形成されていない共通腔 common cavity 型と呼ばれているものである。聴力は表 2 に示すように不良なものが多いが，意外にも中等度の低下にとどまるものもある。

次いで，蝸牛低形成型では聴力低下は軽度のものが多いことがわかる。

C の蝸牛の異常を伴わない半規管・前庭の内耳

奇形は，表2に示したように，蝸牛形態が正常であるために，聴力低下も軽度なものが多いが，一方，高度な難聴を伴うこともある。

Dの前庭水管拡大を伴う奇形では聴力低下は軽度なものから高度まで幅広く分布し，変動性であり，また，低音域の気骨導差の存在が認められることが多い。このために聴力レベルの割に補聴効果が出やすいことが特徴的な点としてあげられる。

II．内耳奇形の型とラセン神経節の残存

内耳奇形でも型によってはラセン神経節がある程度数は形成されていることは組織学的にも確認されている。Jackler ら[4]は共通腔型の2耳，蝸牛低形成の2耳，Mondini 型奇形の1耳の計5耳に初めて単チャンネル方式人工内耳を埋め込み，音知覚を生ず得ることを確認した。以来，多チャンネル方式の人工内耳についても内耳奇形で有効であることが多数報告されている。

Schmidt[5]によれば，正常人のラセン神経節数は25,000から35,000であるのに対し，Mondini 型内耳奇形では7,677から16,110，平均11,478であったと報告している。さらにラセン神経節は共通腔奇形や蝸牛軸形成不全の奇形ではその耳胞壁に存在していると報告した。したがってこれらの内耳奇形でも必ずしも人工内耳が無効ではないと考えられる。ただし，周波数情報を十分に伝えるには，蝸牛の回転がある程度形成されている奇形，すなわちMondini型奇形の方が共通腔型奇形よりも聴取成績の点では有利であろう。Michel型奇形および蝸牛無形成の場合にはラセン神経節の形成もなく，人工内耳の適応とはならない。

III．前庭水管拡大症(Large vestibular aqueduct syndrome，以下LVASと略す)

1．概　説

1978年にValvassoriとClemis[6]によって報告された内耳奇形であり，内リンパ嚢と前庭水管の拡大を特徴とする。CT，MRIなど画像診断が先天性難聴児の画像診断に用いられるようになってから発見頻度は増え，内耳奇形の中でも最も頻度の高い奇形であるとされている[7]。

側頭骨の高分解能CTでは拡大した前庭水管が

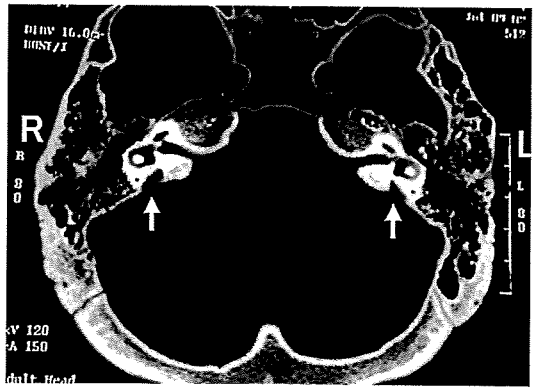

図3　前庭水管拡大症のCTスキャン
矢印：拡大した両側の前庭水管を示す。

前庭に直結している所見が認められる(図3)。合併する奇形として，半規管の異常，拡大した前庭，Mondini奇形の合併などがある。

2．症　状

小児期に発症して，時に頭部の外傷を契機として，めまい発作を反復しながら次第に進行し，ついには高度難聴となる難治性疾患として知られている。

3．遺伝学的異常

本疾患は常染色体劣性遺伝であり，Abe ら[8]は7番染色体長腕（7q31）に原因遺伝子が存在することを報告した。この領域は先天性感音難聴と甲状腺腫を伴うPendred症候群の原因遺伝子であるPDS遺伝子の存在する領域であるが，Usami ら[9]はPDS遺伝子が同時にLVASの原因遺伝子であることを明らかにし，両者はPDS遺伝子変異による表現型が異なる一連の疾患群であることを報告した。

4．聴覚検査所見

46～65％に小児期の難聴があり，15～25％に低音域の気骨導差の存在が認められるという[10]。低音域における気導骨導差の存在に対しては明白な機序は不明であるが，内リンパ圧の上昇によるアブミ骨の可動性の低下も一因として推測される。

内耳障害の機序としては拡大した前庭水管を通

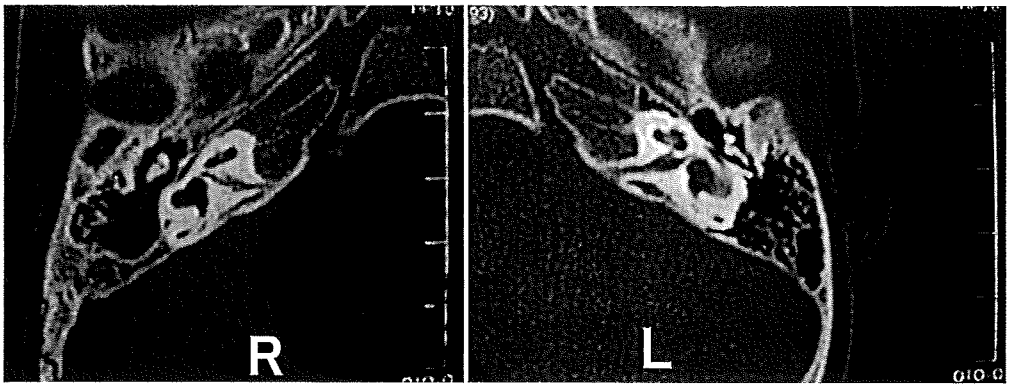

図 4　両側内耳道狭窄症の側頭骨 CT
右滲出液の貯留，外側半規管の奇形および両側内耳道の狭窄が認められる。

じて脳圧が内耳に伝わり，内耳の症状を誘発するものと考えられる。悪化に頭部外傷が契機となることはこれを裏付ける。時には膜迷路が破綻し，急激な聴力低下をきたし，最終的にはコルチ器の障害をきたすものと推測される。しかし，進行性の内耳障害をきたす他の因子の存在も否定できず，複数の機序が存在する可能性もある。

5．治　療

急性増悪時には突発性難聴に準じた副腎皮質ステロイド投与が行われ，ほとんどの例で奏効する。通常，1〜2 週間の投与で回復することが多い。しかし，頻回のめまい発作例では副作用のために投与を中止せざるを得ない例も多い。また長期的には次第に聴力が悪化するのを阻止できない場合が多く，最終的に人工内耳の適応となる例も多い。

これまでに LVAS 症例の拡大した内リンパ嚢を充填する手術が行われて，これを無効とする報告[10]と，有効とする Wilson ら[11]，内藤ら[12]の報告がある。

われわれは，乳突洞削開後に細い硬性内視鏡を硬膜と側頭骨の間に入れて空間を確保し，内リンパ嚢が側頭骨内に入ってゆく部位を確認し，そこでチタン製クリップを用いて内リンパ嚢をクリッピングする新しい術式を考案した[13,14]。本術式は内リンパ嚢の切開を行わずに済み，また圧迫による内リンパ圧の急激な上昇も避けられ，内耳に対する侵襲がこれまでの術式に比べて少なく，聴力保存については内リンパ嚢充填術よりも小侵襲のクリッピング術が有利であった。進行性に 80 dB 程度まで悪化し，かつ，保存治療で改善が期待できなくなった場合には外科的療法の 1 つとして試みられて良いと考える。

Ⅳ．内耳道奇形

1．概　説

ときに先天奇形あるいはクモ膜嚢胞による内耳道拡大が認められることがあるが，この場合，聴力は正常であることが多い。

先天性の内耳道狭窄は蝸牛前庭神経の形成不全が二次的に内耳道の狭窄を引き起こすと考えられている[15]。両側内耳道狭窄症は極めて稀である[16]。両側内耳道狭窄症例の側頭骨 CT を図 4 に示す。

2．内耳道狭窄症の聴覚検査所見

ほとんどの例で聴神経の低形成に伴う高度難聴が認められる。基本的に両側内耳道狭窄症は画像から診断がなされることがほとんどであり，最終的に人工内耳の適応決定，有効性予測の判断が問題となる。

画像診断で高度の両側内耳道狭窄症例あるいは内耳奇形が認められる症例に対して，われわれが行う聴覚検査ならびに画像診断のフローチャートを図 5 に示す。

BOA，COR の聴性行動反応検査，ABR，聴性定常反応検査（ASSR：auditory steady-state response）などの検査で聴覚反応がある程度認められた場合，一般的には純音聴力検査の閾値が100 dB未満で補聴器装用閾値が60 dB未満であれば補聴器の適応である。一方，高度難聴でも聴覚反応がわずかにあり，かつMRIで蝸牛神経が明らかに確認できれば，電気刺激 ABR（EABR）を施行せずとも人工内耳適応ありとして問題ない。

しかし，聴覚反応がほとんどなく，さらにはMRIで聴神経が同定困難である場合には最終的には全身麻酔下で鼓膜から電極刺入し，これによる電気刺激 ABR（electrically induced ABR：EABR）を行うことで適応を決定する。高橋，熊川ら[17]は EABR で反応が認められた両側内耳道狭窄症例に人工内耳埋め込み手術を行い，その有効性を報告した。

V．当院の高度難聴乳幼児の精密聴力検査システム

現在，難聴が疑われる乳児・幼児では，通常は聴性行動反応聴力検査（BOA），条件詮索反射聴力検査（COR），聴性脳幹反応検査（ABR）を行う。しかし行動観察方式の問題点として適応年齢，再現性，左右別の閾値測定が困難，重複障害児における正確さの問題がある。また ABR の問題点としてクリック音を使うために低音域の聴力が反映されず，補聴器の装用効果を予測しにくいという難点がある。このため対象児の年齢が小さくなればなるほど，耳鼻咽喉科医は難聴の程度と聴力像の提示に難渋しているのが現状である。

また，画像検査も必要であり，各検査毎に通院と睡眠剤の投与を行うのは，患児，両親，医師のいずれにとっても負担であった。

そこで，われわれは乳幼児の精密聴力検査のシステムとして，周波数特性を持ち，かつ他覚的な聴力検査である蝸電図検査（electrocochleography：ECoG），聴性定常反応検査（ASSR）に加えて，岬角電気刺激による EABR と CT，MRI も組み合わせて，短期間の入院で乳幼児の検査を効率的に行うシステムを考案し，同一小児群を対象にした検討で以下の結果を得た[18]。

1）鼓室内誘導法による ECoG と ASSR 検査を行うことで ABR 無反応耳の 82％において低音域周波数の域値測定が可能となった。

2）鼓室内誘導法による ECoG は ASSR よりも反応閾値は 5～15 dB 低く，その差は低音域ほど大きく，ECoG の反応検出率は ASSR の2倍高かった。

3）EABR は聴覚反応の得られない内耳道狭窄例など蝸牛神経の形成不全が考えられる例ではきわめて有効な人工内耳の適応決定手段と考えられた。

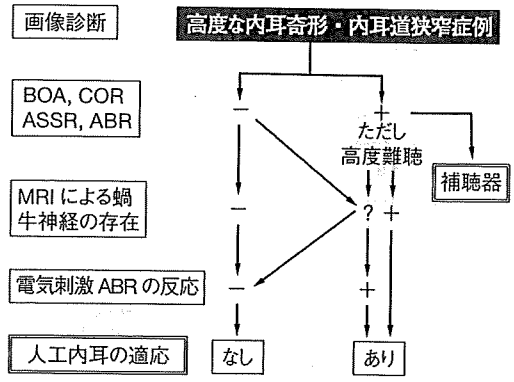

図 5　高度な内耳奇形・内耳道狭窄症の検査フローチャート

おわりに

内耳奇形の診断には聴覚検査のみならず，画像検査，遺伝学的検査も併せて行い，EABR 検査が人工内耳の適応と有効性を予測する上で有用であることを述べた。

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JOHNS
特集●自分でやろう聴覚検査●

聴覚検査トピックス

聴性脳幹インプラントに必要な聴覚検査

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● Key Words ●聴性脳幹インプラント，蝸牛神経核，EABR●

はじめに

聴神経由来の高度感音難聴に対しては人工内耳も効果がなく，これまで外科的治療は困難であった。しかし最近脚光を浴びているのが，聴性脳幹インプラント（auditory brainstem implant，以下ABIと略す）である。これは延髄での聴覚ニューロンの中継核である蝸牛神経核を直接に電気刺激して聴覚を取り戻す人工臓器である。

本稿では，ABIの適応決定に必要な聴覚検査，術中モニタリング，術後のマッピングと評価に必要な聴覚検査について述べる。

Ⅰ．聴覚伝導路の機能解剖

聴覚伝導路の基本的走行を図1に示した。内耳の蝸牛で音は振動から電気的な信号に変換され，同側延髄の中継核である蝸牛神経核（cochlear nucleus：CN）に届く。ここから同側と対側の上オリーブ核に分かれて，外側網体を上行し，中脳の下丘（inferior colliculus：IC）にて中継され，内側膝状体を通って，皮質聴覚野に伝えられる。ここで，ABIはCN上に置かれる。ABIは左右いずれのCN上に設置されても伝導路が交叉し，両側側頭葉に信号が伝えられるので言語の優位半球については考慮しなくともよい。

さらにCNよりも中枢のIC上に電極が設置されるのがauditory midbrain implant（AMI）もすでに試みられている。

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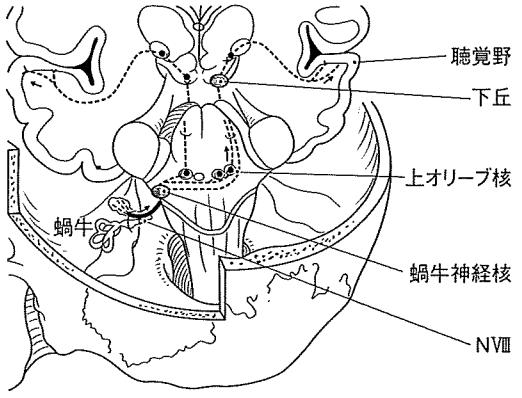

図1 聴覚伝導路の機能解剖

Ⅱ．ABIのシステム

ABIはロサンゼルスにあるHouse耳科学研究所の脳外科医Hitselbergerによって考案され，1979年に両側の聴神経腫瘍を有する神経線維腫症第2型の患者に第1例目の埋め込み手術が行われた[1]。

ABIは当初，単チャンネルであったが，その後，人工内耳の改良とともに多チャンネル化が図られた。現在，Cochlear社製Nuclues 24 ABI（24チャンネル）とMED-EL社製Combi 40＋ABI（12チャンネル）の2種類がある。現在Cochlear社製Nucleus 24 ABIシステムは1999年4月にヨーロッパでの認可を，2002年にアメリカFDAの認可を受けた。MED-El Combi 40＋ABIは，2003年4月にヨーロッパでの認可を受けた。わが国では，まだ認可されていない。

装置のおおまかなシステムは人工内耳と同様であるが，人工内耳が蝸牛に埋め込まれるのに対し，

ABI は CN の表面に置かれる。このため，人工内耳のリング状電極と異なり，Cochlear 社製 Nuclues 24ABI の先端電極は 3×8 mm の長方形で，ここに 22 個のディスク状電極が配列している。一方，MED-El Combi 40＋ABI の先端電極は 5.5×3.0 mm で 12 個の活性電極と 1 個の不活性電極が並んでいる（図 2）。

蝸牛神経核内においても神経細胞は周波数にしたがって tono-topical に配列しており，電極ごとのピッチ弁別が可能である。しかし，その配列は蝸牛ほど整然としたものではなく，さらに深度における周波数配列も存在するという特徴がある。このため，刺激電流量により同一チャンネル内でも自覚的ピッチが変化し，音質そのものが変わるので，人工内耳と比べて，ピッチランキングが難しい[2,3]。

Ⅲ．ABI の適応基準

対象疾患は両側の聴神経腫瘍である。最近の適応拡大として，Colletti ら[4]は先天的な内耳，聴神経の形成不全の小児に ABI を埋め込んでいる。その他，外傷で聴神経が切断された場合，あるいは人工内耳の手術が無効あるいは埋め込み不可能な両側内耳の完全骨化なども適応としている。

Ⅳ．術前検査と適応の決定

1．純音聴力検査，語音聴力検査，補聴器適合評価

アメリカの House 耳科学研究所では，NF2 の症例で一側の聴力が残っていても，手術時の経済的・肉体的負担を減らすために，聴神経腫瘍摘出時にそちら側に同時に ABI を運び込み，将来の失聴に備えるという適応を認めていることから，聴覚的な適応基準は定められていない。しかし，人工内耳よりも劣る成績と，個人輸入による患者の経済的負担（装置代のみで約 280 万円）を考えれば，やはりすでに両側 90 dB 以上の高度難聴があり，かつ補聴器の装用効果が乏しい例を適応とすべきであろう。現在の ABI の語音聴取成績を考慮すれば補聴器装用下での最高語音明瞭度が 10％未満の例を選択することが望ましいと考える。

聴神経腫瘍が摘出された場合にも，聴神経が解剖学的にも機能的にも保存された場合には高度難聴となっても人工内耳治療によって良好な語音聴取能が得られることがすでに報告されている[5]ので，この場合には，成績の良好な人工内耳治療を優先すべきである。この決定には，術中の EABR での反応があることが条件となる。

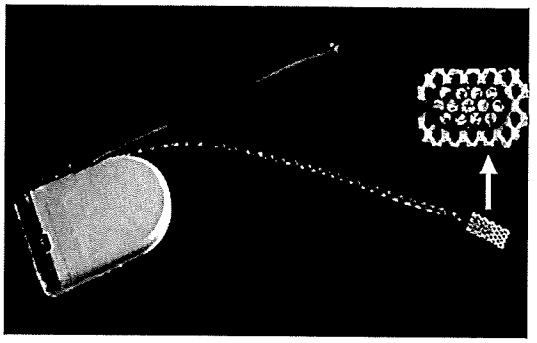

図 2　MED-EL Combi 40＋ABI の電極

大きな腫瘍による脳幹の変形が強い例では，段階手術として電極埋め込みを考慮した方が術後の脳の変形が戻る段階での電極位置の相対的ズレを防ぐためによい場合もある[6]。また患者の全聾としての実体験に伴う障害受容を待ってから手術を行う方がその後の治療リハビリがスムーズに運ぶ。慌てずに，ABI 適応を決定すべきであると考える。

2．プロモントリーテスト（岬角電気刺激検査）

外耳道から鼓膜を穿刺し，蝸牛表面（岬角）に電極を留置し，ここから電気刺激することで，聴神経の機能残存を判断する。筆者らはこれを半定量的検査と捉えている[7]。

当院の 4 例目の ABI 埋め込み例は細菌性髄膜炎により両側内耳が骨化し，他院でプロモントリーテストが陰性であり，かつ同意の下に人工内耳を埋め込まれたが，術後まったく音知覚が得られなかった。当院で ABI 術中に採取された第Ⅷ脳神経の病理組織学的な検査結果では，神経鞘の膨化および断裂，軸策の消失，神経萎縮の像が認められ，人工内耳が無効であった原因が確認された。Gantz ら[8]も同様に蝸牛骨化があり正円窓刺激でも無反応であった 2 例に人工内耳埋め込み術を行ったが，言語聴取可能レベルまで回復しなかったことを報告

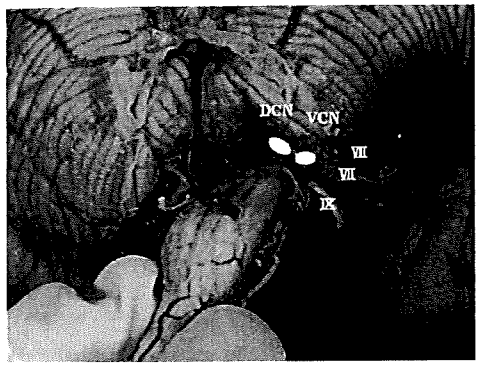

図 3　右側の蝸牛神経核とその付近の解剖
小脳を挙上し，第Ⅳ脳室底を斜め上から見る。
DCN：蝸牛神経背側核，VCN：同腹側核，Ⅶ～Ⅸ：各脳神経

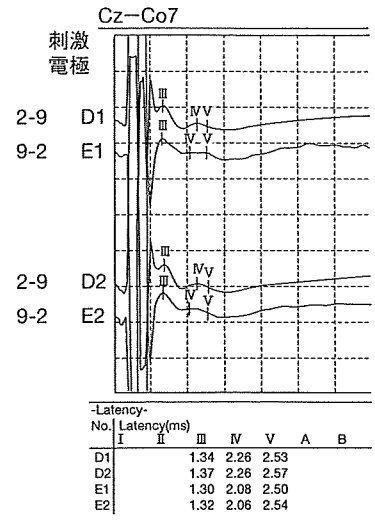

図 4　ABI 埋め込み術中の EABR 波形
Ⅲ波以降の記録と最小の筋原性反応が適切な部位の有用な決定因子である。

している。

人工内耳適応基準ではプロモントリーテスト陰性であることが必ずしも適応禁忌を意味しないとされてはいるが，以上のように，髄膜炎後などで蝸牛の骨化があり，かつ，プロモントリーテスト特に正円窓刺激でも陰性例では人工内耳を勧めるにあたっては十分な説明と同意が必要である。人工内耳が適応されない場合の救済手術として ABI がある。

3．ABR と EABR

対象疾患が NF2 で，すでに聴力が低下している例であれば，通常のクリック音刺激による ABR で反応は得られない。2 のプロモントリーテスト時に鼓室内電極から矩形波電流（持続時間 0.1 ms，強さ 1～2 mA）を流し，刺激と反対側の耳前部電極と頭頂部電極間で EABR を記録する。聴神経が機能している場合には通常Ⅲ波～Ⅳ波が記録され，主観的な音知覚を他覚的に評価できる利点がある[9]。

Ⅴ．術中検査

1．ABI 埋め込み手術と臨床解剖

髄液は第Ⅳ脳室から外側陥凹 lateral recess を通ってクモ膜下腔に流出する。CN は lateral recess の底部で延髄外側に位置し，背側蝸牛神経核 DCN と腹側蝸牛神経核 VCN の 2 つからなる（図 3）。その大きさは CN 全体で幅 2 mm，長さ 12 mm である。第Ⅳ脳室底での DCN は正中溝 median sulcus から 6 mm 外側に位置している。ただし適切な位置確認には後述するように電気刺激によるモニタリングが重要である。

CN へのアプローチ方法としては，経迷路法と後頭下法の 2 つの方法がある[10]。電極を置く位置は背側 CN 側に置くのがよい。その理由は，腹側 CN 上ではⅦ，Ⅸ，下小脳脚などの非聴覚刺激が起きやすいこと，Ⅷ神経切断後に神経根が変性しやすいためである[11]。

2．術中 EABR モニタリング

あらかじめ顔面筋の筋電図による第Ⅶ神経のモニタリングと，外喉頭筋の針電極によるⅨ・Ⅹ脳神経のモニタリングを行っておく。これは腫瘍摘出による麻痺の予防と CN 以外の神経核の電気刺激を検出するために必要である。

さらに電極が適切な位置にあるか否かを決定するのに，術中の電極の電気刺激による EABR モニタリングを行う。人工内耳では EABR のⅡ波以降が記録されるが，ABI では蝸牛神経核とそれ以降の中枢聴覚路が刺激されることによって，EABR の第Ⅲ波以降が誘発される（図 4）。

MED-EL ABI では 4ch のダミー電極 placing