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Hearing impairment in WBN/Kob rats with spontaneous diabetes mellitus

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Summary The inner ear of spontaneously diabetic WBN/Kob rats was functionally and morphologically examined in order to elucidate the relationship between diabetes mellitus and hearing impairment. At 3 months of age, WBN/Kob rats were non-diabetic, and their hearing function was normal. At 6–7 months of age, they showed decreased glucose tolerance and an increasing tendency toward urinary excretion of glucose without high plasma concentration of glucose, and were therefore judged to be pre-diabetic. They also displayed a significant elevation of hearing threshold in the auditory brainstem response, but showed little morphological and histochemical changes in the inner ear. At 12–13 months of age, they were spontaneously diabetic and showed a more apparent elevation of hearing threshold in auditory brainstem response than that in pre-diabetic animals. In addition, they displayed a marked decrease in the number of spiral ganglion cells and oedematous changes in the stria vascularis. The stria vascularis also showed a decrease in the intensity of staining with some lectins, i.e., wheat germ agglutinin, succinylated wheat germ agglutinin, *Soranum tuberosum* lectin, and concanavalin A. In conclusion, hearing impairment is induced by diabetes in the WBN/Kob rats first as an elevation of hearing threshold along with glucose intolerance; secondly, as a decrease in the number of spiral ganglion cells; and thirdly, as oedematous change of the stria vascularis with decreased intensity of lectin staining. [Diabetologia (1995) 38: 649–655]

Key words Diabetes mellitus, hearing impairment, WBN/Kob rats, inner ear, lectins.

Although diabetes mellitus has been investigated both clinically [1–8] and pre-clinically [9–13] in association with hearing loss, the developmental mechanism of hearing impairment in diabetes has not been fully elucidated mainly because of difficulties in functional and morphological examinations simultaneously on human subjects. However, we studied WBN/Kob rats which are derived from Wistar rats [14] and develop diabetes spontaneously due to endocrine-exocrine pancreatic insufficiency [15–19]. They begin to excrete odorous urine abundantly at the age of about 9 months, and thereafter show hyperglycaemia and marked intolerance to glucose. Since they can survive for a long time after the onset of diabetes without insulin treatment, they are a pertinent animal model for analysis of various aspects of diabetes [20, 21]. In the present study, therefore, male WBN/Kob rats with spontaneous diabetes underwent functional, morphological, and histochemical examinations to determine the effects of diabetes on auditory function.

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Abbreviations: ABR, Auditory brainstem response; WGA, wheat germ agglutinin; sWGA, succinylated wheat germ-agglutinin; STL, *Soranum tuberosum* lectin; BSL-II, *Bandeiraea simplicifolia* lectin-II; VVA, *Vicia villosa* agglutinin; ConA, concanavalin A; PHA-E, *Phaseolus vulgaris* erythro-agglutinin; PHA-L, *Phaseolus vulgaris* leucoagglutinin; ABC, Vectastain avidin-biotin complex.

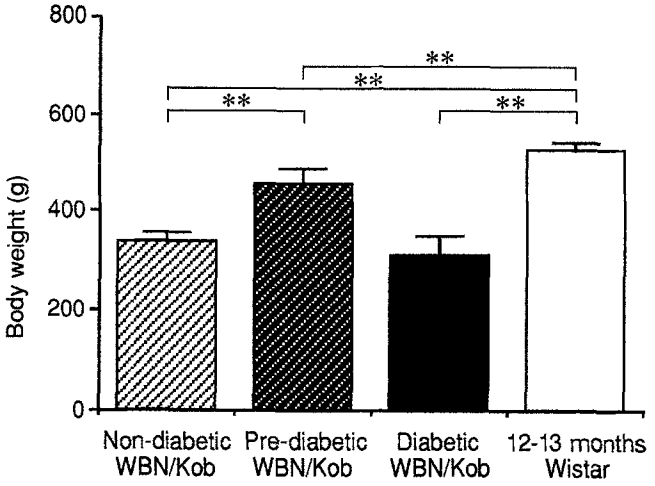

Fig. 1. Average body weight of male non-diabetic, pre-diabetic and diabetic WBN/Kob rats, and of male Wistar rats at 12–13 months of age. Each column represents the mean ± SD ($n = 5$). Significant difference from the non-diabetic WBN/Kob rats or the control Wistar rats ** $p < 0.01$

Materials and methods

Animals. Twenty WBN/Kob and ten Wistar male rats were obtained at 1 month of age from Shizuoka Laboratory Animal Center (Shizuoka, Japan) and kept for 2 to 12 months. The animals were individually housed in stainless steel cages (310 × 270 × 175 mm) and fed standard rat chow (MB-3, Funabashi Farm, Japan) and tap water ad libitum in an animal room with a ventilation capacity of 15 exchanges of room air per hour under controlled temperature (23 ± 2 °C), controlled relative humidity (55 ± 10 %), and a 12-h light/dark cycle (lights on 07.00 to 19.00 hours).

Reagents. Nine biotinylated lectins, wheat germ agglutinin, succinylated wheat germ agglutinin, *Soranum tuberosum* lectin, *Bandeiraea simplicifolia* lectin-II, *Vicia villosa* agglutinin, jacalin, concanavalin A, *Phaseolus vulgaris* erythroagglutinin, and *Phaseolus vulgaris* leucoagglutinin and Vectastain avidin-biotin complex kits were obtained from Vector Laboratories, Inc. (Burlingame, Calif., USA). These lectins bound specifically with the cochlea of the normal Wistar rats in our preliminary examinations (data not shown).

Clinical examinations. Clinical signs, body weight, urinary volume, and plasma and urinary glucose levels (hexokinase method, autoanalyzer Cobas Fara; Roche, Basel, Switzerland) were measured in each rat at intervals of 1 or 3 months throughout the experimental period. One week before killing, the animals were treated orally with glucose at a dose of 2 g/kg to determine plasma glucose concentration at 30, 60, and 120 min after administration for ascertaining glucose tolerance.

Auditory brainstem response (ABR). ABR was recorded in a quiet room in order to estimate the hearing threshold of WBN/Kob rats aged 3, 6–7, and 12–13 months and of Wistar rats aged 12–13 months. Under pentobarbital sodium (40 mg/kg body weight, i.p.) anaesthesia, which has no effect on ABR patterns [22, 23], the animals were kept warm at a rectal temperature of about 38 °C [24, 25], and elicited their ABR to record its pattern with a signal processor (7S-12, NEC San-ei, Tokyo, Japan). Click stimuli of alternating polarity were generated by the signal processor under the conditions of 10 Hz and 0.1-ms duration. Clicks were monaurally delivered through hollow earbars inserted in the right and left external auditory meatuses of the rats. Small enamel-covered stainless wire electrodes (120-μm in diameter) were set subcutaneously at three sites: an active electrode (+) at the vertex, a reference electrode (−) at the base of the stimulated ear, and a ground electrode at the neck.

Histopathological examinations. A few days after the recording of ABR, the animals were transcardially perfused with physiological saline followed by Bouin's fixative under pentobarbital sodium anaesthesia (50 mg/kg body weight, i.p.). After decapitation, the temporal bones including the inner ear were dissected out and immersed in the same fixative overnight. The specimens were decalcified in Plank-Rychlo's solution [26] and embedded in paraffin by routine procedures. Paraffin sections were cut serially at 4 μm thick, deparaffinized with xylene, and stained with haematoxylin and eosin (H.E.) or processed for lectin staining. H.E.-stained sections were examined morphometrically with an image analyser (Luzex 3; Nireco, Tokyo, Japan) to measure the gross area of spiral ganglion cells.

Lectin-histochemical examinations. In the lectin staining, serial sections were incubated separately with nine biotinylated lectins for about 16 h at 4 °C. After incubation, lectin-binding sites were visualized by the Vectastain ABC kit. Control lectin stainings were performed by preabsorption of each lectin with a sufficient amount of its corresponding inhibitory sugar [27] or by the use of phosphate-buffered saline to replace the biotinylated lectins or ABC for the confirmation of the specificity of lectin bindings.

Statistical analysis

The data were statistically analysed by Student's *t*-test. A difference from the respective control was regarded as statistically significant at the $p < 0.05$ level.

Results

Body weight and clinical findings. The average body weight of the WBN/Kob rats was significantly higher just before the onset of diabetes, i.e., at the age of 6–7 months, than at the age of 3 months, but markedly decreased at the age of 12–13 months to slightly lower than that at the age of 3 months (Fig. 1). We defined the WBN/Kob rats aged 3, 6–7 and 12–13 months as non-diabetic, pre-diabetic and diabetic, respectively, according to the clinical findings described in the following, and used the Wistar rats at the age of 12–13 months as the control.

The plasma concentration of fasting glucose was significantly higher in the diabetic WBN/Kob rats than that in the non-diabetic (5.8 ± 0.6 mmol/l) or pre-diabetic (6.4 ± 0.4 mmol/l) WBN/Kob rats and attained a value of over 30 mmol/l. In the glucose tolerance test, the plasma levels of glucose and the rate of its disappearance from the plasma in the non-diabetic WBN/Kob rats were similar to those in the control Wistar rats. The plasma glucose levels were higher in all the pre-diabetic WBN/Kob rats than in the non-

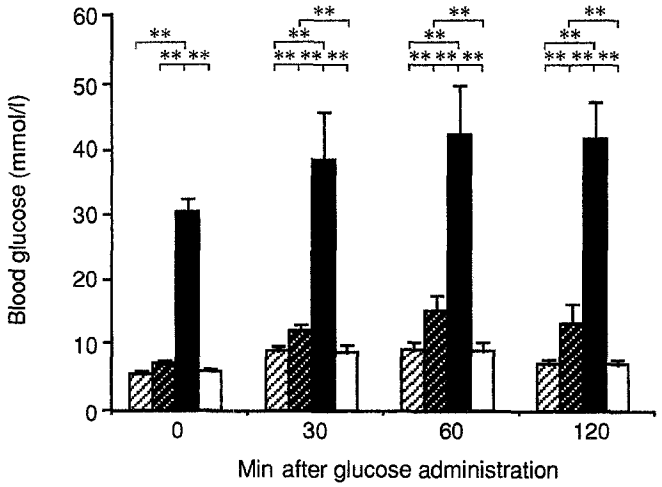

Fig. 2. Oral glucose tolerance test in male non-diabetic (▨), pre-diabetic (▨) and diabetic (■) WBN/Kob rats, and in male Wistar rats (□) at 12–13 months of age. Each column represents the mean ± SD ($n = 5$). Statistical significance in the non-diabetic, pre-diabetic and diabetic WBN/Kob rats, and the control Wistar rats ** $p < 0.01$

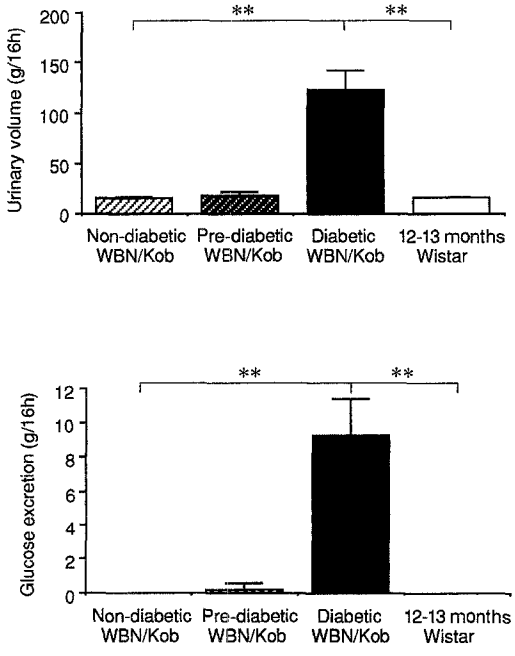

Fig. 3. Average urinary volume and glucose excretion in male non-diabetic, pre-diabetic and diabetic WBN/Kob rats, and in male Wistar rats at 12–13 months of age. Each column represents the mean ± SD ($n = 5$). Significant difference from the non-diabetic WBN/Kob rats or the control Wistar rats ** $p < 0.01$

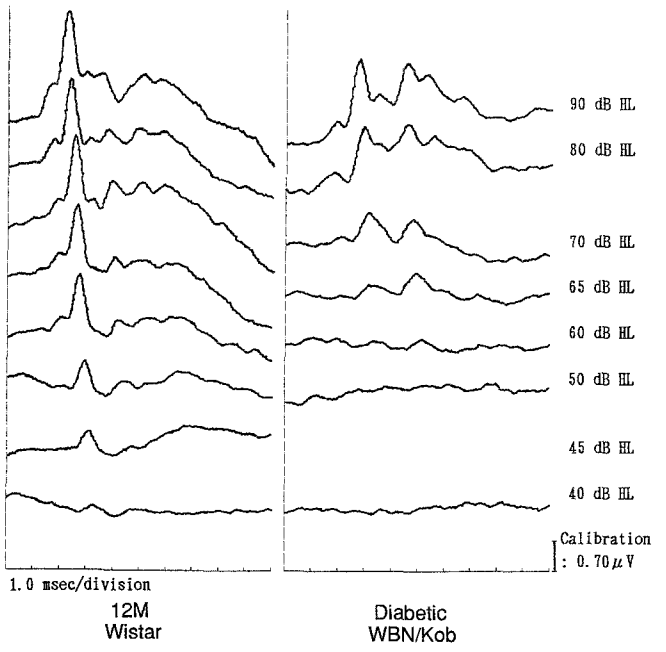

Fig. 4. Typical ABR patterns (right ear) from a control Wistar rat at 12 months of age (hearing threshold: 45 dB HL) and from a diabetic WBN/Kob rat (hearing threshold: 65 dB HL). HL, Hearing level

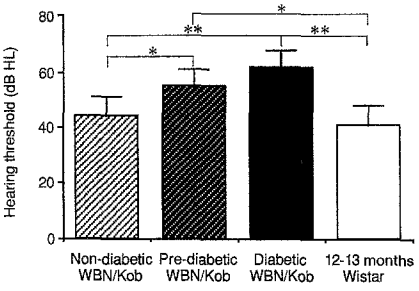

Fig. 5. Average hearing thresholds (right ear) in male non-diabetic, pre-diabetic and diabetic WBN/Kob rats, and in male Wistar rats at 12–13 months of age. Each column represents the mean ± SD ($n = 5$). Significant differences from the non-diabetic WBN/Kob rats or the control Wistar rats * $p < 0.05$; ** $p < 0.01$

diabeticWBN/Kob rats at 30, 60, and 120 min after oral administration of glucose (2 g/kg), although the rate of its disappearance from the plasma was 2.2 mmol/30 min and comparable to that of the non-diabetic WBN/Kob rats (2.3 mmol/30 min) or the control Wistar rats (1.9 mmol/30 min). The plasma glucose levels were five to six times higher in the diabetic WBN/Kob rats than in the non-diabetic WBN/Kob rats. In the diabetic WBN/Kob rats, the plasma glucose levels showed no difference between 30 and 120 min after glucose administration because of the retardation in its disappearance from the plasma. These results are summarized in Figure 2.

The urinary volume and glucose excretion showed significant and marked increases in the diabetic WBN/Kob rats in comparison with those in the non-diabetic WBN/Kob rats, while the glucose excretion was only low in the pre-diabetic WBN/Kob rats (Fig. 3).

Hearing threshold in ABR. The typical patterns of the click stimuli in the diabetic WBN/Kob rat and the control Wistar rat are recorded as shown in Figure 4. Five to six peaks were observed in the ABR patterns

Table 1. Histopathological findings on the cochleae in WBN/Kob and Wistar rats

Strain	Age (months)	No. of animals	Spiral ganglion	Stria vascularis
			Decrease in number of ganglion cells	Oedema of intermediate cells
WBN/Kob	3 (non-diabetic)	5	– (72.0 ± 4.8)[a]	–
	6 ~ 7 (pre-diabetic)	5	– ~ ± (69.2 ± 10.5)	–
	12 ~ 13 (diabetic)	5	± ~ ++ (41.5 ± 10.2)	± ~ +
Wistar	12 ~ 13	5	– (73.5 ± 2.6)	–

[a] Ratio of area of ganglion cells to Rosenthal's canal. Values are % mean ± SD.
Grade of changes: –, no lesion; ±, slight; +, moderate; ++, severe

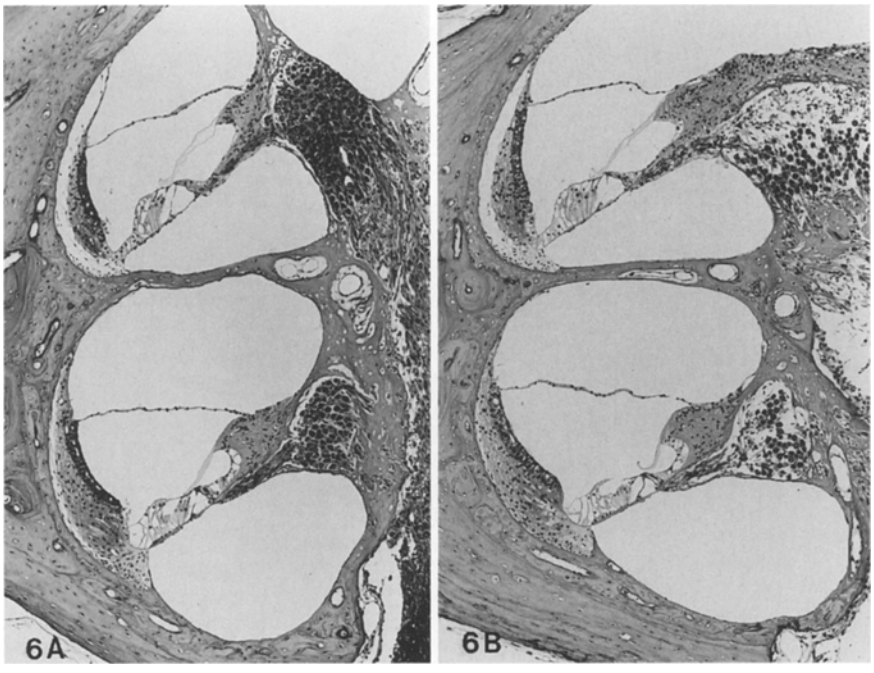

Fig. 6 (A, B). Inner ear of a control male Wistar rat at 12 months of age (**A**) and an age-matched diabetic male WBN/Kob rat (**B**). Loss of spiral ganglion cells and oedema of the stria vascularis are evident in the diabetic male WBN/Kob rat. H. E., × 80

of each animal at the sound pressure levels over the auditory threshold. As the amplitude of the second peak was the highest among them, the hearing threshold was defined as the level at which the second peak could be distinguished.

The average hearing thresholds of the right ear of the non-diabetic, pre-diabetic and diabetic WBN/Kob rats were 44 ± 7, 55 ± 6, and 62 ± 6 dB HL (hearing level), respectively, and were similar to those of the left ear. The threshold was significantly higher in pre-diabetic and diabetic WBN/Kob rats than that in the non-diabetic WBN/Kob rats. The average hearing threshold was comparable in the non-diabetic WBN/Kob rats to that in the control Wistar rats. These results are shown in Figure 5.

Histopathological findings. The histopathological findings on the cochleae in WBN/Kob and Wistar rats are summarized in Table 1. H.E.-stained sections of the inner ear of a control male Wistar rat and a diabetic male WBN/Kob rat are illustrated in Figures 6 and 7. Histopathological changes were focused on the spiral ganglion and the stria vascularis in the cochleae of the diabetic WBN/Kob rats. They involved a decrease in the number of the spiral ganglion cells and oedematous change in the stria vascularis. The latter change was predominant in the intermediate cells. One out of five pre-diabetic WBN/Kob rats showed a slight decrease in the number of the spiral ganglion cells. In contrast, the inner and outer hair cells showed no histopathological changes in any WBN/Kob rat.

Lectin-histochemical findings. The lectin-binding patterns of the cochleae in WBN/Kob and Wistar rats are summarized in Tables 2 and 3. The intermediate cells

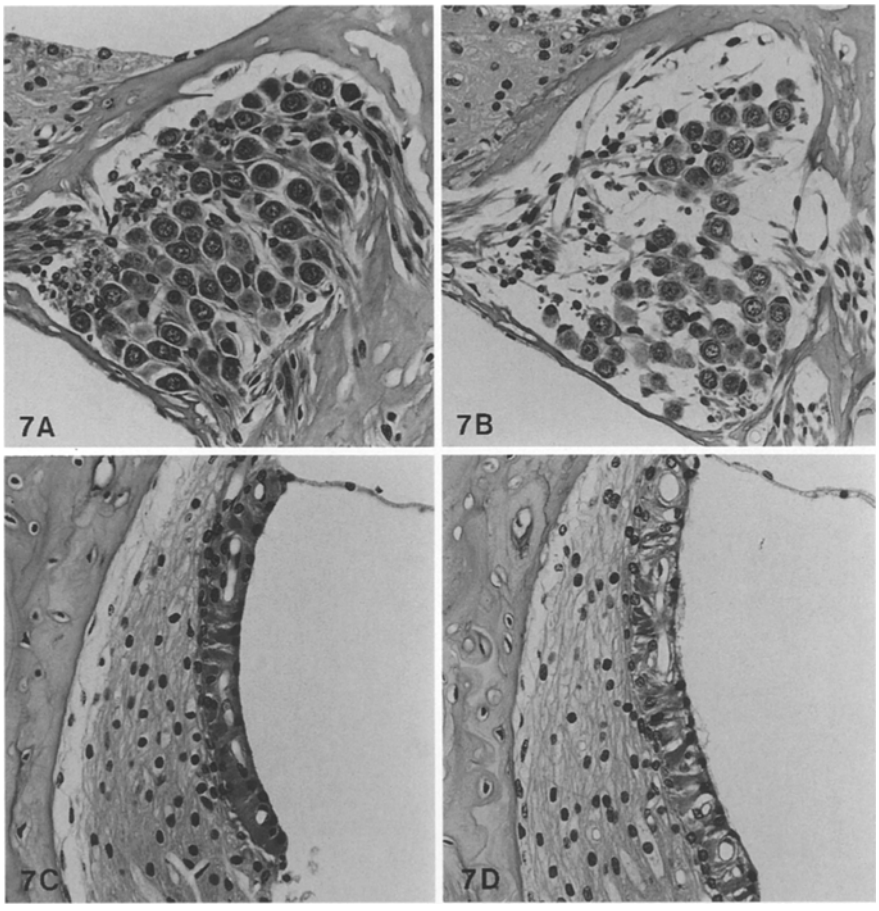

Fig. 7 (A–D). High magnifications of the spiral ganglion (**A, B**) and stria vascularis (**C, D**) of the same animals as shown in Figure 6. There are no abnormalities in the control male Wistar rat (**A, C**). The decrease in the number of spiral ganglion cells (**B**) and oedematous change in the intermediate cells of the stria vascularis (**D**) are evident in the diabetic male WBN/Kob rat. H. E., × 330

Table 2. Lectin binding patterns of the cochleae (stria vascularis) in WBN/Kob and Wistar rats

Strain	Age (months)	No. of animals	Marginal cells	Intermediate cells			
			VVA	WGA	s-WGA	STL	Con A
WBN/Kob	3 (non-diabetic)	5	± ~ +	+	++	++	+ ~ ++
	6 ~ 7 (pre-diabetic)	5	± ~ +	± ~ +	++	++	+ ~ ++
	12 ~ 13 (diabetic)	5	± ~ +	±	± ~ +	± ~ +	±
Wistar	12 ~ 13	5	± ~ +	± ~ +	+ ~ ++	++	+ ~ ++

Grade of staining: ±, faint; +, moderate; ++, intense

of the stria vascularis in the diabetic WBN/Kob rats were more weakly stained with WGA, s-WGA, STL, and Con A lectins than those in the non-diabetic or pre-diabetic WBN/Kob rats. Lectin stainings of the stria vascularis with STL are compared between a control male Wistar rat and a diabetic male WBN/Kob rat in Figure 8. On the other hand, the superficial or marginal cells were specifically stained with VVA throughout all the non-diabetic, pre-diabetic and diabetic WBN/Kob rats and control Wistar rats. There were no differences in the staining intensity of Con A, jacalin, PHA-E, and PHA-L in the spiral ganglion cells, or that of BSL-II in the outer hair cells between the pre-diabetic and diabetic WBN/Kob rats.

Discussion

With respect to the changes in body weight, WBN/Kob rats showed an ordinary and progressive increase in body weight until 6–7 months of age (pre-diabetic stage), but a decrease at the age of 12–13 months (diabetic stage). They did not develop diabetic obesity.

In the oral glucose tolerance test, the fasting plasma glucose levels, glucose tolerance, and urinary glu-

Table 3. Lectin binding patterns of the cochleae (spiral ganglion) in WBN/Kob and Wistar rats

Strain	Age (months)	No. of animals	Ganglion cells			
			Con A	Jacalin	PHA-E	PHA-L
WBN/Kob	3 (non-diabetic)	5	+ ~ ++	± ~ +	± ~ +	±
	6 ~ 7 (pre-diabetic)	5	+ ~ ++	± ~ +	± ~ +	±
	12 ~ 13 (diabetic)	5	+ ~ ++	± ~ +	±	±
Wistar	12 ~ 13	5	+ ~ ++	± ~ +	± ~ +	± ~ +

Grade of staining: ±, faint; +, moderate; ++, intense

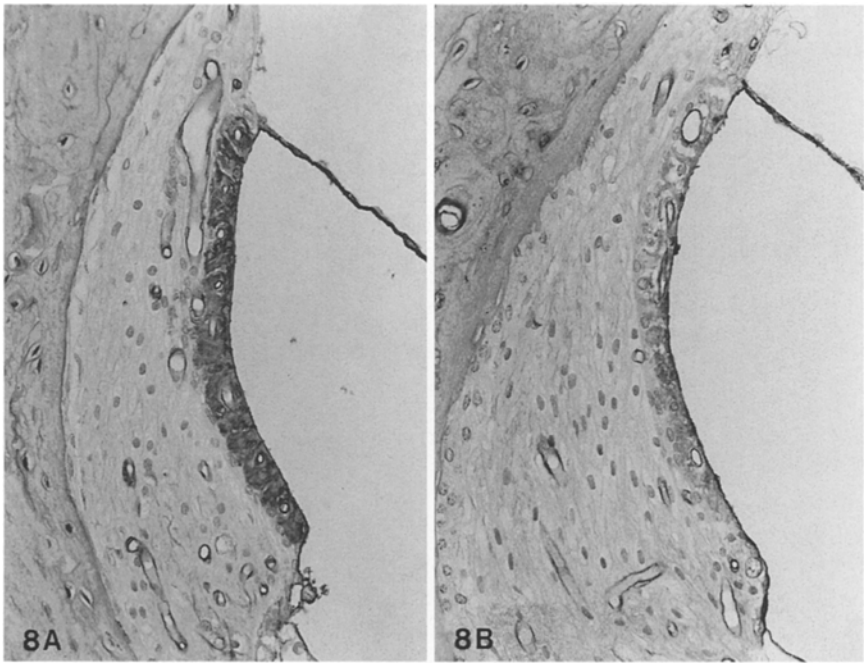

Fig. 8 (A, B). Stria vascularis of a control male Wistar (**A**) and diabetic male WBN/Kob rat (**B**). In the control male Wistar rat, marginal and intermediate cells in the stria vascularis are stained intensely with STL, while in the diabetic WBN/Kob rat, intermediate cells are only faintly stained with STL. × 330

cose were normal in the non-diabetic WBN/Kob rats aged 3 months when compared with those in the control Wistar rats. WBN/Kob rats aged 6–7 months were almost normal in their fasting plasma glucose levels, but were slightly intolerant of glucose and negative or falsely positive for urinary glucose, and regarded as pre-diabetic. On the other hand, WBN/Kob rats aged 12–13 months were significantly high in their fasting plasma glucose levels, apparently intolerant to glucose, positive for urinary glucose and polyuria, and were concluded to be diabetic.

The auditory function was examined by the ABR. The hearing thresholds of non-diabetic WBN/Kob rats were comparable to those of the control Wistar rats. In contrast, those of pre-diabetic and diabetic WBN/Kob rats were significantly high depending on the severity of the diabetes.

Although histopathological changes such as thickening of the vessel wall in the stria vascularis and spiral ligament [1, 4], hair cell loss in the organ of Corti, and spiral ganglion atrophy [3] have been reported in the inner ear of human subjects, it is not always clear whether these changes are primarily due to diabetes. There are too many complicating factors to resolve this problem [6, 7].

In the present study, the decrease in the number of spiral ganglion cells and the oedematous change in the intermediate cells of the stria vascularis were observed in the inner ear of the diabetic WBN/Kob rats. The latter was similar to the reversible and oedematous changes in the stria vascularis of guinea pigs treated with diuretics such as furosemide, which is potentially ototoxic [28–30]. In addition, one of five pre-diabetic WBN/Kob rats revealed a slight decrease in the number of spiral ganglion cells without changes in the stria vascularis. This animal showed the highest elevation of hearing threshold in the same age group. These findings may suggest that the hearing impairment is primarily caused by the decrease in the number of spiral ganglion cells and secondarily enhanced by the oedematous change in the stria vascularis.

In lectin-histochemical examinations, the spiral ganglion cells in the diabetic rats were comparable to those in the non-diabetic and pre-diabetic rats in terms of staining intensity of Con A, jacalin, PHA-E, and PHA-L. These data suggest that the spiral ganglion cells are normal in function in spite of the decrease in their number. On the other hand, the intermediate cells of the stria vascularis were more weakly stained with WGA, s-WGA, STL, and Con A in the diabetic rats than those in the non-diabetic and pre-diabetic rats. The decrease in the intensity of staining may imply the dysfunction of the stria vascularis.

In summary, the present results indicate that the onset of hearing impairment is already evident at the pre-diabetic stage. The hearing impairment is further aggravated by the extensive decrease in the number of the spiral ganglion cells and the oedematous change in the intermediate cells of the stria vascularis. These findings suggest a significant relationship between diabetes and hearing impairment in this animal model.

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