Cranial Nerve Dysfunction in Conscious Galactosemic Rats as Measured by the Auditory-Evoked Brainstem Response (43033)

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Abstract. **The purpose of this study was to extend our previous work with the auditory-evoked brainstem response and determine whether galactosemia would produce a functional neuropathy similar to that previously seen in streptozocin-induced diabetic rats. Sprague-Dawley male rats implanted with cortical electrodes received either normal chow (n = 17) or a 50% galactose diet (n = 17) for 5 weeks. Peak II latency of the auditory-evoked brainstem response, interpreted as a functional measure of the auditory nerve (VIII cranial) in rats, was significantly prolonged in galactose-fed rats relative to controls ($P < 0.05$). These results demonstrate a functional deficit in the auditory nerves of galactosemic rats. The deficit in the auditory-evoked brainstem response of galactosemic rats is similar to our previous finding in streptozocin-induced diabetic rats.**
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One metabolic consequence of diabetes is elevated levels of tissue glucose, particularly in those tissues which do not require insulin for glucose uptake (lens, nerve, retina, kidney). In these tissues the excess glucose is converted to its sugar alcohol (sorbitol) by aldose reductase, the first enzyme in the polyol pathway. The diabetes-induced metabolic alteration in the peripheral nerve, retina, lens, and kidney has been implicated in tissue functional abnormalities and the pathogenesis of diabetic complications (1, 2).

Nerve conduction velocity has been used as a measure to assess nerve function and evaluate the efficacy of new therapeutic agents for the treatment of diabetic neuropathy (3–9). Pharmacologic studies in rats have shown that inhibition of aldose reductase prevents both the accumulation of polyols and the slowing in nerve conduction velocity (3–6). The fact that beneficial effects can be achieved in diabetic rats by structurally different aldose reductase inhibitors strongly indicates an association between aldose reductase activity and the development of diabetic neuropathy, and further suggests that aldose reductase inhibitors may be beneficial in the treatment of diabetic neuropathy in man.

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Recently, we began using the auditory-evoked brainstem response (ABR) to assess nerve function in diabetic rats (10). ABR latencies were delayed in streptozocin (STZ)-induced diabetic rats and tolrestat, an aldose reductase inhibitor, prevented the delay in the ABR. These findings support the use of the ABR as a functional measure of a diabetes-induced nerve dysfunction and imply that the polyol pathway is involved in this neuropathy.

The role of the polyol pathway in diabetic neuropathy can also be examined in galactosemic animals. In this model, galactose added to the diet results in the accumulation of galactitol within nerves and other tissues via the polyol pathway (11). An accumulation of neural galactitol has been associated with reduced nerve conduction velocity in rats (12, 13) which was reversible on withdrawal of galactose from the diet (12). Therefore, similar to diabetes, polyol accumulation and associated alterations occur in the galactosemic rat, although without the confounding factors due to the administration of STZ.

The purpose of this study was to extend our previous work with the ABR and determine whether galactosemia would produce a functional neuropathy similar to that previously seen in STZ-induced diabetic rats (10).

Materials and Methods

Sprague-Dawley male rats (Charles River, Wilmington, MA) weighing between 300 and 400 g (ap-

proximately 70–90 days of age) were anesthetized (pentobarbital sodium, 40 mg/kg ip) and implanted with cortical electrodes (coordinates 3 mm anterior, 3 mm right; 0 mm anterior, 9 mm right; 3 mm posterior, 3 mm left (ground) of bregma). Following recovery from surgery the animals were housed in individual cages with free access to water and food. Animals were assigned by body weight to either a control group (n = 17) or a group receiving a 50% galactose diet (n = 17). Animals were maintained on their respective diets for the 5 weeks of the study.

Detection of Cataracts. The development of cataracts was monitored by slit lamp microscopy after the instillation of atropine (1%) in the eyes of each animal. The lenticular changes were classified according to the method of Sippel (14) as modified previously (15). Lenses were scored as follows: 0 = normal lens; 1 = faint peripheral opacity; 2 = irregular peripheral opacity and slight involvement of the central portion of the lens; 3 = irregular opacity involving the entire lens; and 4 = macroscopically visible opacity. Scores from two observers were pooled, and data were reported as the "opacity index" (16), i.e., the average score for all lenses in each group.

ABR Recording. The methods and procedures for recording the ABR were as previously reported (10), with minor modifications. Binaural open-filed ABR were elicited by 1000 repetitive clicks (0.1-ms duration, 11 Hz, 85 dB). The recorded potentials were differentially amplified (×10,000, bandpass 3 HZ-10 kHz; Grass P511k) and processed further by digital filtering (low pass at 3 kHz).

Peak latencies were measured post hoc using the cursor-driven waveform analysis functions. Latency values were normalized as the change relative to baseline. The effect of galactosemia on the ABR latencies was determined by a one-way analysis of variance and a one-tailed t test. The significance level was set at $P < 0.05$ (a) and $P < 0.01$(b).

Results

General. There were no overt behavioral or physical changes in any of the rats except for differences in body weight. The mean body weight of the control rats increased gradually during the study, whereas that of the galactose-fed rats remained unchanged (Table I). This difference was significant for all time periods after baseline ($P < 0.05$).

Lenticular Changes. Progressive development of lenticular opacities was observed in all of the animals fed the galactose chow (Fig. 1). After 2 weeks of galactose feeding, 59% of the animals had developed peripheral lens opacities. By the end of 5 weeks of galactose feeding, all animals had peripheral opacities, while 82% of the animals had partial or complete involvement of the central portion of the lens. Macroscopically, visible opacities were not observed in any of the animals. No lenticular changes were observed in any of the animals fed control chow.

ABR Peak Latencies. Binaural, open-field ABR were reproducibly recorded from conscious, unrestrained animals. Typical ABR records had eight peaks in the first 6 msec of the recorded response (Fig. 2). Comparison between the galactose-fed and control groups indicated no significant differences between Peak I latency over the duration of the study (Table II). In contrast, Peak II latency of the galactose-fed group was significantly greater than that of controls for all 4 weeks following the introduction of the diet. In addition, there was no significant difference between the latency of Interpeak II and IV. Collectively, these results indicate that the transmission deficit of the ABR in galactose-fed rats occurs between the generator sites for Peaks I and II, while no deficit is apparent after Peak II as indicated by evaluation of Interpeak latency II-IV.

Discussion

In this study all of the galactose-fed animals developed lenticular opacities, thus confirming the onset and progression of the galactosemic state. The rate of cataract development was slower than that observed previously in rats fed 50% galactose (16). The slower rate of cataract development may be due to the use of larger rats (300–400 g body wt range), with lower food intake.

Our results demonstrate a nerve dysfunction in galactosemic rats, as measured by a relative increase in Peak II latency. In addition, there was no deficit seen in Peak I or after Peak II (Interpeak latency II-IV). This clearly indentifies the dysfunction as occurring at the generator site for Peak II. These findings are in agreement with our previous study which showed that Peak II latency increased in STZ diabetic rats (10).

Although the generator site for Peak II was once thought to be the cochlear nucleus, more recent evidence suggests that it is the auditory nerve (17, 18). In rats, it has been shown that following exposure to misonidazole neurotoxicity, Peaks I and II were the only peaks not affected (17). In the same study, histologic examination showed consistent necrosis in the cochlear nuclei, leading the authors to suggest that the generator for Peak II is the auditory nerve. In man, recent evidence also suggests that Peak II is generated by the auditory nerve rather than the cochlear nucleus (18). In this light, it is reasonable to interpret the increased Peak II latency observed in this and the previous study (10) as slowed nerve transmission in the VIII cranial nerve.

The latency of Peak II of the ABR of control animals decreased over the 4 weeks of the study. A similar change in Peak II latency was seen in our previous study (10). A decreased latency, which reflects an increase in nerve conduction velocity, is not an

Table I. Body Weight for Control and Galactose-Fed Rats[a]

Group	*n*	Weeks of galactose feeding					
		0	1	2	3	4	5
Control	17	442 ± 5	451 ± 7	488 ± 6	509 ± 6	535 ± 7	560 ± 7
Galactose-fed	17	442 ± 9	442 ± 8[b]	430 ± 7[b]	433 ± 7[b]	436 ± 7[b]	439 ± 7[b]

[a] Values are expressed as mean ± SE.
[b] $P < 0.05$ as compared with the control.

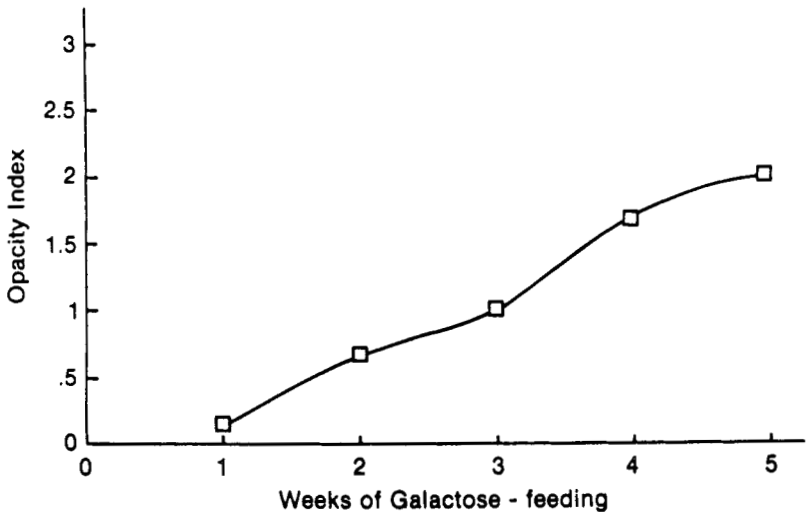

Figure 1. Mean stage of cataract development in galactosemic rats (n= 17) as measured by the "opacity index."

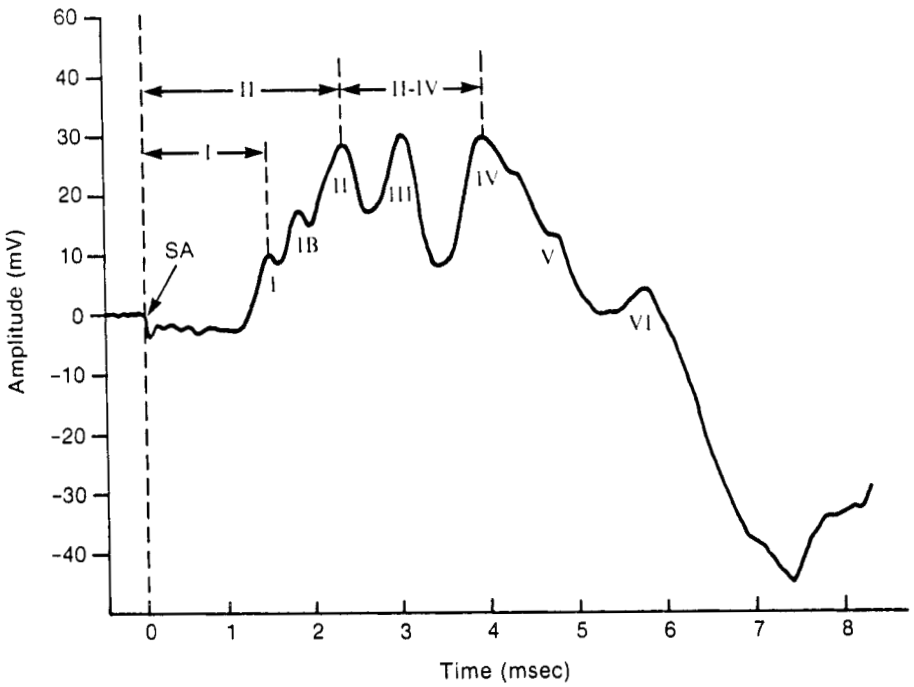

Figure 2. An averaged ABR recorded from a conscious normal rat. Identified peaks were labeled according to the nomenclature previously used in this laboratory (10). The measurements used for analysis included the latencies for Peaks I and II and the interpeak latency between peaks II and IV. SA, stimulus artifact.

Table II. Mean Change (±SE) in ABR Latency (μsec) from Baseline

	Week			
	1	2	3	4
Peak I				
Control	18.7 ± 16.1	27.1 ± 15.5	36.7 ± 13.2	14.3 ± 10.4
Galactose-fed	−1.5 ± 18.0	17.1 ± 17.0	5.3 ± 16.5	14.7 ± 16.1
Peak II				
Control	−48.9 ± 26.7	−90.0 ± 17.7	−92.5 ± 22.3	−64.4 ± 24.2
Galactose-fed	85.0 ± 26.1[a]	51.4 ± 23.0[a]	92.5 ± 61.2[b]	82.9 ± 73.4[b]
Interpeak II–IV				
Control	0.0 ± 36.8	0.0 ± 63.7	0.0 ± 33.4	−51.4 ± 52.9
Galactose-fed	−45.0 ± 80.3	−40.0 ± 84.6	−22.9 ± 127.6	−116.7 ± 138.8

[a] $P < 0.05$ as compared with control.
[b] $P < 0.01$ as compared with control.

unexpected finding. It is accepted that in rats, nerve conduction velocity increases to a maximum at about 300 days of life (19). The rats used in this study were approximately 200 days old, and therefore would be expected to show a further increase in conduction velocity.

In this study the galactosemic rats had lower body weights relative to controls. It could be argued that the galactose diet retards maturation, which could account for the observed slower nerve conduction velocity. In another study, it was shown that ABR latencies of STZ-induced diabetic rats are increased relative to age-matched controls, but are similar to weight-matched controls (20). This led the authors to speculate that diabetes may slow the maturation of the auditory system. Our earlier study, however, suggests that this is not the case (10). We have shown that in tolrestat-treated STZ-induced diabetic rats, body weight was significantly reduced but nerve function, as measured by the ABR, was normal. Therefore, we conclude that the reduction in body weight is not causative of the increased latency of Peak II.

There are several clinical reports of deficits in the ABR of diabetics (21–27). Some of these studies found changes in the early conponents of the ABR (21, 22, 24, 26), while others described deficits in more central components (25, 27). These functional reports support studies which have shown cranial nerve demyelination (29–31) and hearing deficits (31, 32) in diabetic patients. Clearly further work is needed to clarify the degree of cranial nerve neuropathy in diabetics.

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1. Greene DA, Lattimer SA, Sima A. Sorbitol, phosphoninositides, and sodium-potassium-ATPase in the pathogenesis of the diabetic complications. N Engl J Med **316**:599–606, 1987.
2. Winegrad AI. Does a common mechanism induce the diverse complications of diabetes? Diabetes **36**:396–406, 1987.
3. Yue DK, Hanwell MA, Satchell PM, Turtle JR. The effect of aldose reductase inhibition on motor nerve conduction velocity in diabetic rats. Diabetes **31**:789–94, 1982.
4. Gillon KRW, Hawthorne JN, Tomlinson DR. Myo-inositol and sorbitol metabolism in relation to peripheral nerve dysfunction in experimental diabetes in the rat. The effect of aldose reductase inhibition. Diabetologia **25**:365–71, 1983.
5. Mayer JH, Tomlinson DR. Prevention of defects of axonal transport and nerve conduction velocity by oral administration of myo-inositol or an aldose reductase inhibitor in streptozotocin-diabetic rats. Diabetologia **25**:433–38, 1983.
6. Tomlinson DR, Moriarty RJ, Mayer JH. Prevention and reversal of defective axonal transport and motor nerve conduction velocity in rats with experimental diabetes by treatment with the aldose reductase inhibitor sorbinil. Diabetes **33**:470–476, 1984.
7. Ward JD. Diabetic neuropathy. Diabetes Annu **1**:288–308, 1985.
8. Judzewitsch RG, Jaspan JB, Polonsky KS, Wienberg CR, Halter JB, Halar E, Pfeifer MA, Vukadinovic C, Bernstein L, Schneider M, Liang KY, Gabby KH, Rubenstein AH, Porte D Jr. Aldose reductase inhibition improves nerve conduction velocity in diabetic patients. N Engl J Med **308**:119–25, 1983.
9. Kikkawa R, Hatanaka I, Yasuda H, Kobayashi N, Shigeta Y, Terashima H, Morimura T, Tsuboshima M. Effect of a new aldose reductase inhibitor (E)-3-carboxymethyl-5[(2E)-methyl-3-phenyl-propenylidene] rhodanine (ONO-2235) on peripheral nerve disorders in streptozotocin-diabetic rats. Diabetologia **24**:290–292, 1983.
10. Notvest RR, Inserra JJ. Tolrestat, an aldose reductase inhibitor, prevents nerve dysfunction in conscious diabetic rats. Diabetes **36**:500–504, 1987.
11. Stewart MA, Sherman WR, Kurien MM, Moonsammy GI, Wisegerhof M. Polyol accumulations in nervous tissue of rats with experimental diabetes and galactosemia. J Neurochem **14**:1057–1066, 1967.
12. Gabbay KH, Snider JJ. Nerve conduction defect in galactose-fed rats. Diabetes **21**:295–300, 1972.
13. Sharma AK, Thomas PK, Baker RWR. Peripheral nerve abnormalities related to galactose administration in rats. J Neur Neurosurg Psych **39**:794–802, 1976.
14. Sippel TO. Changes in water, protein, and glutathione contents of the lens in the course of galactose cataract development in rats. Invest Ophthalmol **5**:568–575, 1966.
15. Simard-Duquesne N, Dvornik D. Galactitol accumulation and

irreversible lens opacities in galactosemic rats. Invest Opthalmol **12**:82–83, 1973.
16. Simard-Duquesne N, Greselin E, Gonzalez R, Dvornik D. Prevention of cataract development in severely galactosemic rats by the aldose reductase inhibitor, tolrestat. Proc Soc Exp Biol Med **178**:599–605, 1985.
17. Edwards MSB, Powers SK, Baringer RA, Jewett DL, Bolger C, Philips TL. Evoked potentials in rats with misonidazole neurotoxicity. I. Brainstem auditory evoked potentials. J Neurooncol **1**:115–23, 1983.
18. Scherg M, von Cramon D. A new interpretation of the generators of BAEP waves I-V: Results of a spatio-temporal dipole model. Electroencephalogr Clin Neurophysiol **62**:290–299, 1985.
19. Birren JE, Wall PD. Age changes in conduction velocity, refractory period, number of fibers, connective tissue space and blood vessels in sciatic nerve of rats. J Comp Neurol **104**:1–16, 1956.
20. Finegold DN, Sabo DL, Tanpowpong K, Mackway AM. Auditory evoked brainstem response in streptozotocin (S) diabetic rats. Diabetes **37**(suppl 1):118A, 1988.
21. Donald MW, Erdahl DLW, Surridge DHC, Monga TN, Lawson JS, Bird CE, Letemendia FJJ. Functional correlates of reduced central conduction velocity in diabetic subjects. Diabetes **33**:627–633, 1984.
22. Fedele D, Martini A, Cardone C, Comacchio F, Bellavere F, Molinari G, Negrin P, Crepaldi G. Impaired auditory brainstem-evoked responses in insulin-dependent diabetic subjects. Diabetes **33**:1085–89, 1984.
23. Harkins SW, Gardner DF, Anderson RA. Auditory and somatosensory far-field evoked potentials in diabetes mellitus. Int J Neurosci **28**:41–47, 1985.
24. Hendriks JJT, De Jong FICRS, Hogenhuis LAH. Investigations of retrochochlear function in diabetic neuropathy. Clin Otolaryngol **10**:51, 1985.
25. Ben-David J, Gertner R, Podoshin L, Fradis M, Pratt H, Rabina A. Auditory brain stem evoked potentials in patients suffering from peripheral facial nerve palsy and diabetes mellitus. J Laryngol Otol **100**:629–633, 1986.
26. Goldsher M, Pratt H, Hassan A, Shenhav R, Eliachar I, Kanter Y. Auditory brainstem evoked potentials in insulin-dependent diabetics with and without peripheral neuropathy. Acta Otolaryngol (Stockh) **102**:204–208, 1986.
27. Khardor R, Soler NG, Good SC, Devlesc HAB, Broughton D, Walbert J. Brainstem auditory and visual evoked potentials in type 1 (insulin-dependent) diabetic patients. Diabetologia **29**:362–365, 1986.
28. Verma A, Gisht MS, Ahaja GK. Involvement of central nervous system in diabetes mellitus. J Neuro Neurosurg Psych **47**:414–416, 1984.
29. Reske-Nielson E, Lundback K, Rafaelson OJ. Pathological changes in the central and peripheral nervous system of young long-term diabetics. Diabetologia **1**:223–241, 1965.
30. Makishima K, Tanaka K. Pathological changes of the inner ear and central auditory pathway in diabetics. Ann Otol Rhinol Laryngol **80**:218–228, 1971.
31. Jorgensen MB, Buch NH. Studies on inner-ear function and cranial nerves in diabetes. Acta Otolaryngol **53**:350–364, 1961.
32. Friedman SA, Schulman RH, Weiss S. Hearing and diabetic neuropathy. Arch Intern Med **135**:573–76, 1975.