AUDITORY BRAINSTEM RESPONSE (ABR) AND EFFECTS OF FUROSEMIDE ON ABR IN CONSCIOUS F344 RATS

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(Received May 7, 1996 ; Accepted June 28, 1996)

ABSTRACT — The present study was conducted to evaluate the variations in the normal auditory brainstem response (ABR) between the F344 and Wistar rats. It was also investigated whether strain differences exist in the ABR profiles of each strain of rat given furosemide, which induced ototoxicity.

The typical waveform of ABRs in the F344 rats were similar to those observed in the Wistar rats. There were no significant differences between the strains in almost all parameters, but peak latencies from P1 to P3 in the F344 rats tended to be longer than those in the Wistar rats.

After a single intravenous injection of furosemide, the minimum effective dosage levels, at which each peak of the ABR disappeared, were 53.3 ± 8.2 and 70.0 ± 8.9 mg/kg in the F344 and Wistar rats, respectively. A single intravenous injection of 100 mg/kg furosemide delayed the reversal of decrease in amplitude, prolongation of peak latency and the reappearance time in F344 rats.

Thus, the normal waveform of ABR was comparable in the F344 and Wistar rats, but a tendency towards a prolongation in each latency was noted in the F344 rat. The results also suggested that the ABR of F344 rats were more vulnerable to furosemide ototoxicity than that of Wistar rats.

KEY WORDS : Auditory brainstem response (ABR), Ototoxicity, F344 rats, Wistar rats, Furosemide

INTRODUCTION

As an experimental animal for ototoxicity studies, guinea pigs have been used generally because their cochlear apparatus is similar to that of humans. However, various rat strains have been used recently in toxicity studies, and it is desirable to select the same strain for ototoxicity studies.

As one of the electrophysiological procedures for assessment of auditory function, the measurement of auditory brainstem response (ABR), a short-latency far-field sensory evoked potential, has been conducted. Yamamoto and Chiba (1984) established a procedure to record the ABR using conscious Wistar rats, and they have applied it to evaluate auditory impairment induced aminoglycoside antibiotics (Yamamoto *et al.*, 1992).

The present study was conducted to clarify normal ABR in conscious Fisher (F344) rats and

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to evaluate the variations in ABR between the F344 and Wistar rats. It was then investigated whether strain differences existed in the ABR profiles of each rat given high doses of furosemide, which induces ototoxicity (Brummett *et al.*, 1975 ; Rybak 1985).

MATERIALS AND METHODS

1. Animals

Six male F344/Jcl rats and 6 male Jcl :Wistar rats (CLEA Japan Inc., Tokyo, Japan) with ages ranging from 28 to 34 weeks, weighing 318–335 g and 420–465 g, were used to detect variations of the normal auditory brainstem response (ABR) and effects of furosemide on each rat strain ABR. A second group from the escalating-dose study to elucidate the minimum effective dose for furosemide ototoxicity was comprised of 6 male F344/Jcl rats and 6 male Jcl :Wistar rats with ages ranging from 25 to 30 weeks, weighing 302–321 g and 400–452 g (CLEA). At the start of the experiments, all animals were about 30 weeks old. Each animal was housed individually in a stainless steel cage in a room with a constant 12 hr light-dark cycle. The room temperature was maintained at $23 \pm 3^\circ\text{C}$ with a relative humidity at $55 \pm 15\%$. The animals were allowed free access to a standard laboratory diet (CE-2, CLEA) and tap water. Their maintenance and the experimental protocols conformed to the Guide for the Care and Use of Laboratory Animals of Takeda Chemical Industries, Ltd.

2. Drug

Furosemide, a commercial product, (Lasix®, Höchst Japan Inc., Tokyo, Japan), each vial containing 20 mg of furosemide, was used. A single escalating dose of furosemide was administered intravenously to each rat via the tail vein every 2 or 4 days. The dosage of furosemide was increased in 10 mg/kg increments from 30 to 100 mg/kg and 50 to 100 mg/kg in F344 and Wistar rats, respectively. According to these results, a single dosage of 100 mg/kg was then administered to assess whether strain differences existed in the ABR profiles in the first pair of groups.

3. Surgery and ABR recording

The animals were anesthetized with ketamine hydrochloride (100 mg/kg, intramuscularly ;

i.m.) and doroperidole (1 mg/kg, i.m.). After anesthesia, a stainless steel screw was placed as an active electrode on the epidural surface of the lower margin of the parietal bone. Another screw as the reference electrode was placed in the sinus of the frontal bone. In addition, a stainless steel wire was inserted into the right temporalis muscle and served as the ground. The electrodes were led to a miniature socket (Ampenol®, Honda Communication MGF Co., Tokyo, Japan) and fastened to the skull with dental cement (Yatapoliset®, Yata Chemical Ind. Co. Ltd., Osaka, Japan). The animals were allowed at least 1 week to recover from the surgery.

For ABR recording, a conscious rat was placed in a sound-attenuated box in an electrically shielded room. A slip ring (Electro-cannula slip ring, Airflyte Electronics Co.) was used to allow the animal to move in a relatively free manner without twisting the cables. ABR recording was started after an adaptation period of 20–30 min before furosemide dosing, during which time the animal became accustomed to the box. The potentials were evoked by 0.5, 1, 2, 4 and 8 kHz tone pips of 90 dB SPL that had 0.25-millisecond exponential rise-fall times, a 10-millisecond duration and a 125-millisecond inter-stimulus interval. A tone pip was presented through a speaker located on the top of the box (about 50 cm in height). One hundred twenty-eight responses to repetitive stimulation were averaged with an evoked response recording system (MEB-5100, Nihon Kodan Co., Osaka, Japan).

4. Normal ABR and effects of furosemide

Cochlear microphonics (CM), peak 1 (P1), peak 2 (P2), peak 3 (P3) and peak 4 (P4) were observed as aspects of the ABR (Fig. 1). The amplitude of CM was measured from peak to peak of the initial wave, and those of P1, P2, P3 and P4 were measured from the baseline to peak of each wave. The latency was measured as the time from stimulus onset to each initial wave peak (Yamamoto *et al.*, 1992).

To clarify the variations in ABR between the F344 and Wistar rats, the P4/P1 amplitude ratio (P4/P1 ratio) and the P1 to P4 interpeak latency (P1–P4 IPL) of each ABR were calculated from amplitude or latency.

Each ABR, evoked by 8 kHz tone pip of 90

F344 rat

Wistar rat

Fig. 1. Typical waveforms of ABR evoked by a 8 kHz tone pip at 90 dB SPL in conscious F344 and Wistar rats. The ABR in the F344 rat was similar to that observed in the Wistar rat.

dBL, was recorded within 5 min after each treatment of furosemide (40–100 mg/kg, in 10 mg/kg steps) in the escalating-dose study. Each dosing was carried out a few days interval. The minimum effective dose was detected by elimination of all potentials in the ABR in each animal. ABR, evoked by 8 kHz tone pip of 90 dBL, was also recorded 1, 5, 10, 20, 40, 80, 160, 320 and 1440 min after dosing of 100 mg/kg furosemide, in order to detect the time (reappearance time) that the detectable level of each wave in rat ABR was restored. The amplitudes and latencies of all waves except for CM were measured and analyzed. However, the effects of furosemide on the amplitude and latency in ABR were assessed using only P4 because the changes in the amplitudes and latencies of all waves except CM were much the same as those in P4.

5. Statistical analysis

The data on the amplitude, peak latency, P4/P1 ratio, P1–P4 IPL and minimum effective dose for furosemide ototoxicity were analyzed as follows. First, the data were tested by F test (Snedecor and Cochran, 1980) for homogeneity of variance between the F344 and Wistar rat groups. If the variances were homogeneous, the Student's t-test was applied and if the variances were heterogeneous, the Aspin & Welch t-test was performed to compare each mean value. All tests were conducted at the 5% and 1% two-tailed probability levels. P-values less than 0.05 were accepted as significant.

RESULTS

1. ABR in conscious F344 and Wistar rats

The typical waveform evoked by a 8 kHz

tone pip at 90 dB SPL in a conscious F344 rat consisted of P1, P2, P3 and P4 waves occurring within 5 msec post-stimulus, following initial deflections corresponding to the cochlear microphonic (CM) (Fig. 1). The ABRs in the F344 rats were similar to those observed in the Wistar rats.

Table 1 shows the changes in the amplitude in each wave. There were no significant differences in each value between the two strains. Over the frequency range of 0.5 to 8 kHz, each amplitude showed a tendency towards an increase, except for that in CM in both strains. However, there was no change in the amplitude of CM waves with increasing sound frequency.

Each peak latency and P1-P4 IPL in the F344 and Wistar rats are shown in Table 2. There were significant differences between the strains in P1 latencies at 2, 4 and 8 kHz, P2 latencies at 1, 2, 4 and 8 kHz, and P3 latency at 4 kHz. These peak latencies in the F344 rats were longer than those in the Wistar rats. For the other peak latencies, there were no significant differences between the strains. Each peak latency in both strains also showed a tendency

towards a decrease with increasing sound frequency.

In addition, there were no significant differences in the P4/P1 ratio or P1-P4 IPL between the F344 and Wistar rats.

2. Effects of furosemide on ABR in the F344 and Wistar rats

From the results in the escalating dose study of furosemide, the minimum effective dosages of furosemide to eliminate all potentials in the ABR were 53.3 ± 8.2 mg/kg in the F344 rats, and 70.0 ± 8.9 mg/kg in the Wistar rats, a significant difference from that in Wistar rats ($p < 0.01$).

After a single intravenous injection of furosemide at the dosage level of 100 mg/kg, which was enough to eliminate each wave, ABRs were recorded 1, 5, 10, 40, 80, 160, 320 and 1440 min after dosing in F344 and Wistar rats. Fig. 2 illustrates the typical changes of ABR at the 8 kHz sound frequency. In both strains, all the peaks of ABR disappeared within 1 min after furosemide dosing, and reversal of the P4 wave also was observed within 20 min after dosing. Twenty-four hours (1440 min) after dosing, waveform alterations had almost disappeared for all

Table 1. Amplitude of each peak (μ V) and P4/P1 amplitude ratio on ABR in conscious F344 and Wistar rats.

Peak or ratio	Strain	Number of animals	Frequency (kHz)				
			0.5	1	2	4	8
CM	F344	6	/	/	1.54 ± 0.54	0.95 ± 0.23	0.94 ± 0.22
	Wistar	6	/	/	1.51 ± 0.18	1.06 ± 0.20	0.86 ± 0.11
P1	F344	6	2.70 ± 1.11	2.70 ± 0.90	3.36 ± 1.03	4.89 ± 1.92	6.71 ± 2.04
	Wistar	6	2.51 ± 0.95	2.95 ± 0.95	4.05 ± 1.64	5.45 ± 2.15	7.97 ± 3.29
P2	F344	6	1.69 ± 0.74	3.63 ± 1.35	3.25 ± 1.25	3.39 ± 1.01	5.26 ± 0.96
	Wistar	6	2.94 ± 1.30	4.61 ± 1.28	3.77 ± 1.89	3.50 ± 1.19	4.74 ± 2.37
P3	F344	6	3.94 ± 0.93	2.91 ± 0.86	4.09 ± 1.43	5.08 ± 1.46	7.39 ± 1.27
	Wistar	6	2.83 ± 0.81	3.52 ± 0.77	4.24 ± 1.47	5.47 ± 1.93	8.07 ± 2.68
P4	F344	6	3.77 ± 0.82	5.44 ± 1.20	5.52 ± 1.86	6.17 ± 1.92	9.38 ± 1.72
	Wistar	6	4.79 ± 1.92	6.04 ± 2.85	7.08 ± 3.00	6.54 ± 3.30	8.88 ± 3.79
P4/P1	F344	6	1.58 ± 0.66	2.08 ± 0.38	1.66 ± 0.32	1.35 ± 0.46	1.45 ± 0.31
	Wistar	6	1.90 ± 0.29	2.04 ± 0.68	1.77 ± 0.32	1.15 ± 0.25	1.16 ± 0.27

/ : Not detected.

ABRs were evoked by 0.5 to 8 kHz tone pips at 90 dB SPL. Each value represents the mean and standard deviation. There were no significant differences in any value between the two strains.

the frequencies, in both strains.

The changes in P4 amplitude after furosemide dosing of 100 mg/kg are shown in Fig. 3. In all frequencies, the P4 amplitude decreased more in the F344 rats than in the Wistar rats from 10 to 40 min after dosing. Thereafter, there were no differences between the strains. A reversal of the P4 amplitude to normal level delayed at a lower frequency of sound stimulation in both strains.

The changes in P4 latency after furosemide dosing of 100 mg/kg are listed in Table 3. For all frequencies of sound stimulation, the P4 latency showed a tendency to be prolonged after dosing in the F344 rats, more so than in the Wistar rats. The P4 latency also tended to be prolonged at a lower frequency of sound stimulation in both strains, and the reversal of this change at 0.5 kHz was not observed in the F344 rats.

The reappearance time of each wave in the furosemide-treated F344 and Wistar rats is given in Table 4. For all frequencies of sound stimulation, the reappearance time of each peak in the

F344 rats except for CM were longer than those in the Wistar rats.

DISCUSSION

There were no significant differences between the F344 and Wistar rats in the waveform of ABR, amplitude of each wave, P4/P1 ratio, latency of CM, P3 and P4, and P1-P4 IPL. However, P1 and P2 peak latencies in the F344 rats were longer than those in the Wistar rats. Chen and Chen (1990) suggested that differences in anatomical factors in different strains of rats might affect peak latencies. Although much of the brainstem physiology is similar, anatomical differences may exist in the brain stems of different strains of rats, e.g., the sizes of the brain stem nuclei, the lengths of the acoustic fiber tracts or the spatial alignment of the neurons (Huang 1980). Thus, based on the present results, it may be possible that the difference in peak latencies is due to an anatomical difference.

In the escalating dose study, a lower mini-

Fig. 3. Alteration of P4 amplitude after dosing of furosemide in F344 and Wistar rats. Each value represents the mean and standard deviation. *, **: Significantly different from values in Wistar rats, $p < 0.05$ and $p < 0.01$, respectively. Number of animals: $n = 6$ at 8 kHz; $n = 5$ at the other frequencies in each strain. Each amplitude decreased more in the F344 rats than in the Wistar rats from 10 to 40 min after dosing.

Table 3. Change in P4 latencies after furosemide dosing in conscious F344 and Wistar rats.

Frequency of tone pip (kHz)	Strain	Number of animals	Time after furosemide-injection (min)									
			0	1	5	10	20	40	80	160	320	1440
0.5	F344	5	4.82±0.15	/	/	/	6.87±0.32	6.37±0.40	5.53±0.77	5.67±0.82	5.29±0.79	5.01±0.18
	Wistar	6	4.77±0.07	/	/	6.41±0.25	6.13±0.51	5.17±0.64	5.22±0.85	4.82±0.10	4.73±0.19	4.73±0.10
1	F344	5	4.74±0.09	/	/	/	5.93±0.08	5.39±0.31	5.03±0.27	5.13±0.35	4.92±0.32	4.78±0.14
	Wistar	6	4.72±0.15	/	/	5.77±0.22	5.31±0.27	4.95±0.27	5.24±0.67	4.77±0.09	4.70±0.07	4.57±0.09
2	F344	5	4.55±0.29	/	/	/	5.60±0.12	5.12±0.18	4.71±0.25	5.05±0.75	4.75±0.48	4.54±0.12
	Wistar	6	4.50±0.09	/	5.16	5.17±0.31	4.70±0.15	4.57±0.08	4.79±0.37	4.81±0.40	4.66±0.27	4.48±0.11
4	F344	5	4.46±0.27	/	/	/	5.23±0.28	4.94±0.11	4.62±0.25	4.77±0.43	4.57±0.33	4.50±0.25
	Wistar	6	4.46±0.12	/	5.04	4.97±0.36	4.65±0.15	4.51±0.13	4.63±0.11	4.71±0.34	4.56±0.22	4.36±0.18
8	F344	6	4.44±0.09	/	/	/	5.26±0.25	4.76±0.19	4.58±0.16	4.70±0.20	4.60±0.23	4.45±0.09
	Wistar	6	4.33±0.12	/	0.88±0.33	4.65±0.17	4.54±0.10	4.42±0.06	4.44±0.12	4.48±0.12	4.46±0.20	4.31±0.10

/ : Not detected.

ABRs were evoked by 0.5 to 8 kHz tone pips at 90 dB SPL. Each value represents the mean and standard deviation. The P4 latency values showed a tendency to be longer in the F344 rats in the Wistar rats after injection.

Table 4. Reappearance time (min) of each wave in furosemide-treated F344 and Wistar rats.

Peak	Strain	Number of animals	Frequency (kHz)				
			0.5	1	2	4	8
CM	F344	6	/	/	40~320	20~80	20~80
	Wistar	6	/	/	10~40	10~160	20~80
P1	F344	6	40~160	20~80	20~80	20~40	10~40
	Wistar	6	10~80	10~80	1~20	5~20	5~20
P2	F344	6	40~160	20~40	20~40	10~40	10~40
	Wistar	6	5~80	5~40	1~20	1~10	1~10
P3	F344	6	20~80	20~80	10~80	10~40	10~40
	Wistar	6	5~40	5~40	1~20	1~20	1~10
P4	F344	6	10~40	10~40	10~40	10~40	10~40
	Wistar	6	5~20	5~10	1~10	1~10	1~10

/ : Not detected.

ABRs were evoked by 0.5 to 8 kHz tone pips at 90 dB SPL. Each value represents the range. Reappearance times were defined as the time from the disappearance of each peak of the ABR to the reappearance of their partial amplitude. Reappearance times of all peaks except for CM were longer in the F344 rats than in the Wistar rats.

imum effective dosage for furosemide ototoxicity was detected in the F344 rats. Furthermore, a single intravenous injection of 100 mg/kg furosemide caused a decrease in the P4 amplitude in the F344 rats that was more remarkable than that in the Wistar rats, and the P4 latency in the F344 rat showed a tendency to be more prolonged than in the Wistar rats. The reversal of each wave to a detectable level in the F344 rats was earlier than in the Wistar rats. Therefore, furosemide affected auditory function in the F344 rats more strongly than in the Wistar rats. Furosemide affects stria vascularis and suppresses acoustic function (Rybak, 1985; Rybak *et al.*, 1991 and 1992). This phenomenon has been suggested to be caused by a mechanism whereby furosemide suppresses the activity of Na⁺ and K⁺-ATPase located on epithelial cells of the stria vascularis (Rybak, 1985), resulting in a decrease in the amplitude of endocochlear potential (EP) and/or cochlear microphonics (CM) (Konishi *et al.*, 1966) and in morphological changes (Comis *et al.*, 1990; Rybak *et al.*, 1991 and 1992).

In the present study, it is suspected that furosemide affected the stria vascularis in the F344 rats more strongly than in the Wistar rats. Strain differences may be present in furosemide uptake into perilymph and/or serum concentra-

tions, because of the differences in the minimum effective dosage for furosemide ototoxicity, and these differences may be critical in explaining the discrepancy in ototoxic effects across rat strains. Further examinations of strain differences, e.g., differences in toxicokinetics or blood-cochlear barrier (Juhn *et al.*, 1985) would be informative.

At the lower frequency of sound stimulation, the reversal in the changes of P4 amplitude and peak latency was observed across strains in the present study. This may be associated with the hearing sensitivity of rats, since the audiogram of rodents exhibits a lesser sensitivity at frequencies lower than 4 kHz (Kelly and Masterton, 1977); the reason for this is unclear.

In conclusion, the normal waveform of the ABR was comparable in the F344 and Wistar rats studied, but a tendency towards a prolongation in each latency was noted in the F344 rat. The results also suggested that the ABRs of the F344 rats were more vulnerable to furosemide ototoxicity than those of the Wistar rats because of the lower minimum effective dosage for ototoxicity and the delay of reversal in the ABR changes in the F344 rats.

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