Pre- and Postovulatory Auditory Brainstem Response in Normal Women

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Abstract Studies with ovarian hormones on auditory brainstem response (ABR) have conflicting reports although women have ABR shorter than men. This study compared ABR between pre- and postovulatory phases of menstrual cycle in consenting 40 healthy female volunteers (age 19 ± 2.35 years). The study was conducted under standard laboratory conditions (room temperature $26 \pm 2^\circ\text{C}$) using Neuropack (Nihon Kohden machine, NM-420S; H636, Japan) and earphone dynamic receiver ELEGA (Type DR-531; no.237, Japan) in sound proof room. Ears were stimulated simultaneously using standard protocol. ABR was recorded in pre- and postovulatory phases. Ovulation was estimated by measuring basal body temperature. The ABR wave latencies (WLs) I, II, III, IV, V and inter-peak latencies (IPLs) I–III, III–V, I–V were compared between the two phases of menstrual cycle using paired t test. The postovulatory phase had shorter WL V (5.71 ± 0.18 ms vs. 5.81 ± 0.19 ms, $p < 0.01$), IPL III–V (1.89 ± 0.16 ms vs. 1.94 ± 0.19 ms, $p < 0.05$), and I–V (3.88 ± 0.16 ms vs. 3.95 ± 0.18 ms, $p < 0.05$) than in preovulatory. Other WLs and IPLs showed decreasing trend in postovulatory phase. ABR is better in postovulatory phase

as compared to preovulatory phase probably due to progesterone hormone which might be involved in modulating auditory hearing pathway at postovulatory phase.

Keywords Menstrual cycle · Hearing · Auditory brainstem response (ABR) · Sensory conduction · Estrogen · Progesterone

Introduction

Auditory brainstem (ABR) responses are signals generated in the auditory nerve and brainstem following an acoustic stimulus. ABR is examined for evaluation of hearing and suspected disorders of the brainstem. Its components are known to vary with different conditions such as age, sex, temperature, drugs, and hearing loss. The ABR wave replicability recorded from females are better defined and have larger amplitude than those of males which is attributed to the sex difference in cochlear response time [1, 2]. High estrogen level, small brain size and shorter auditory pathway [3, 4] in female seems to be responsible for better hearing in female than in male as suggested by the findings of most of the studies. Others suggest that higher estrogen level delays in peak III appearance, due to brainstem synaptic impairment (via ischemic changes), resulting in a higher incidence of Bell's palsy during pregnancy [5].

It is well known that the level of sex hormones, estrogen and progesterone in female changes with the change in phases of menstrual cycle. Wide variation is observed in different parameters of ABR at different phases of menstrual cycle. There is increase in wave latencies (WLs) of all waves in midcycle and decrease in latencies in mid-luteal phase with the smallest latencies occurring during menstruation, Interpeak latencies (IPLs) and amplitude

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follow the same trend [4–9]. Estrogen has been suggested to be responsible for delaying the conduction by influencing GABA release at the level of polysensory association areas of brain [6]. However, better wave replicability with decreased WLs and IPLs during follicular and periovular phase than during the luteal phase has been shown by other studies [10]. Moreover, ABR recorded on hormone replacement therapy (HRT) treated individuals and pill users has shown shorter WLs and IPLs values during periovular phase with respect to the luteal and follicular phase suggesting hearing enhancing effect of estrogen and hearing attenuating effect of progesterone [11–19].

It is proposed that estrogen and progesterone modulate secretion of GABA in auditory pathway in a counter regulatory fashion [20]. Estrogen may interact with the surface membrane receptors or ion channels to change the excitability of nerve cells. It is proposed to be an allosteric antagonist of NMDA 'R' and could be protective against excitotoxicity of glutamate by simply delaying the conduction in auditory pathway [21]. It is also reported that progesterone is vital for brain cells based on the fact that progesterone levels are so high during pregnancy helping in brain cell maturation, repairing and maintaining proper functions of neurons [22]. Ovarian steroids have been reported to have influence on cochlear blood flow and potentially hearing functions too [23].

Our hypothesis is that these hormones have role in modulating hearing pathway and therefore ABR recorded at pre- and postovulatory phases of menstrual cycle show significant difference in WLs and IPLs of ABR. The purpose of this study was to investigate whether

the conduction as measured by ABR is better in pre-ovulatory or postovulatory phase of menstrual cycle. To elucidate the influence of estrogen and progesterone on ABR we considered that peak estrogen is present in blood before ovulation and peak progesterone is present in blood after ovulation in normal menstruating women. See Fig. 1.

Methods

This study compared ABR between pre- and postovulatory phases of menstrual cycle in forty consenting healthy female volunteers of age 16–35 years and BMI <25 kg/m². Volunteers having normal gynecological history and hearing assessed by tuning fork test (Rinne's test and Weber's test) were included in the study. Women receiving steroid hormones or therapy for depression, having history of head injury, stroke, or heart attack were excluded. Moreover, pregnant or lactating mother and ladies with drug dependence in whom steroid hormone secretion or neuronal plasticity are likely to be affected were also excluded. Females enrolled did not report any dysendocrinism or metabolic neoplastic pathologies and without any hearing loss. Harmful doses of alcohol or nicotine were confirmed by scoring two WHO scoring systems; Fragestrom test for nicotine dependence (score ≥ 8) [24] and alcohol use disorder identification test (AUDIT score ≥ 8) [25].

Volunteers were instructed to note down their menstrual calendar for at least 2 months along with their basal body temperature (BBT) for detecting the day of ovulation. Females having constant days of menstrual calendar for those two months were recruited for the study. In all the women meeting the selection criteria, ears was stimulated simultaneously using standard protocol for ABR.

Electrode Placement

Electrodes were placed according to the 10–20 international system of EEG electrode placement. All the metal ornaments of the subject were removed from the body parts before recording to minimize disturbances while recording. The skin at the point of placement of electrodes was cleaned. By using electrode paste or conducting jelly the recording (active) electrodes were placed on both the ear mastoid process, reference electrode at vertex Cz and the ground electrode was placed at front of the vertex that is at the Fz according to the 10–20 international system of EEG electrode placement.

Fig. 1 Variation in estrogen and progesterone hormone along the length of menstrual cycle. (Source: Guyton and Hall: Textbook of medical Physiology, 11th edition)

ABR Recording Procedure

The ABR was recorded using Neuropack (Nihon Kohden machine, NM-420S; H636, Japan) and earphone dynamic receiver ELEGA (Type DR-531; no.237, Japan) in a sound proof room with three surface electrodes with impedance $<5 \text{ k}\Omega$ using both channel 1 and 2. The machine filter was set at low cut 100 Hz and high cut at 3 kHz electrical interference from the surrounding electrical appliances. Acoustic stimulus was delivered at the rate of 10 Hz having 65 db SL intensity. The number of epochs per trial was 4,000. The type of stimulus used was of alternating phase. The data were digitized over an epoch duration or analysis time of 10 ms.

The ABR was recorded in both pre- and postovulatory phases of normal menstrual cycle of 28 ± 7 days. Time interval between two measurements was kept similar as far as possible within subjects. Ovulation was estimated by measuring BBT. Most of the volunteers were called 2–4 days prior to expected day of ovulation for recording preovulatory ABR while most were called 9–11 days after ovulation for recording their postovulatory ABR, according to their menstrual cycle as shown in the Table 1. Waves were obtained from both ears stimulated simultaneously. We stimulated both ears simultaneously because each ears cannot be considered as an independent samples. In hearing pathway there is no distinct anatomical separation of auditory nerve fibers for each ears after synapsis in superior olivary nucleus to cerebral cortex. Moreover most of the nerve fibers from the respective ear crosses and synapse to the contralateral superior olivary nucleus and ascends to the auditory cortex. So the acoustic stimuli delivered to one ear can alter the function of both the ipsilateral and contralateral hearing pathway. Hence both ears are stimulated simultaneously to obtain combined effect of both the ears on delivery of a stimulus and to minimize the chances of error that can occur on the waveforms of ABR recorded. So altogether we analyzed 80 observations (40 preovulatory phase data points and 40 postovulatory phase data points).

The ABR WLs I, II, III, IV, V and IPLs I–III, III–V, I–V was recorded and compared between the two phases of

Table 1 Selecting days for maximum constancy of auditory brainstem response (ABR) in subjects with different days of menstrual cycle

Menstrual cycle in days	Ovulation day	Day for preovulatory ABR testing	Day for postovulatory ABR testing
25	11	7 or 8 or 9	20 or 21 or 22
28	14	10 or 11 or 12	23 or 24 or 25
30	16	12 or 13 or 14	25 or 26 or 27
35	21	17 or 18 or 19	30 or 31 or 32

Table 2 General characteristics of the subjects, $n = 40$

Variable	Mean $\pm$ SD	Range
Age	19.25 ± 2.35 years	16–26 years
Height	1.57 ± 0.05 m	1.44–1.67 m
BMI	$20.07 \pm 2.36 \text{ kg/m}^2$	13.79–24.89 kg/m^2
Age at menarche	12.7 ± 0.97 years	10–14 years

menstrual cycle. The outcome data obtained were expressed as mean $\pm$ SD and compared using paired t-test.

Results

Forty healthy female volunteers with normal menstrual cycle and without any hearing problems were included in the present study. The general characteristics of the subjects including age at menarche are presented in Table 2.

On stimulation of both ears, the postovulatory phase had shorter WL- V (5.71 ± 0.18 ms vs. 5.81 ± 0.19 ms, $p < 0.01$), IPL III–V (1.89 ± 0.16 ms vs. 1.94 ± 0.19 ms, $p < 0.05$), and I–V (3.88 ± 0.16 vs. 3.95 ± 0.18 ms, $p < 0.05$) than in preovulatory phase. However, other WLs and IPLs showed generally decreased values in postovulatory phase as compared to preovulatory phase as presented in Table 3.

Discussion

There are number of laboratory findings suggesting that there are latency changes in the waves of ABR that are related to fluctuating hormone levels during different phases of menstrual cycle. Many postmenopausal women as well as those who have irregular menstrual period,

Table 3 Comparison of WLs and IPLs of auditory brainstem response in preovulatory and postovulatory phase of the both ears on simultaneous stimulation, $n = 40$

Waves & interpeak latencies (IPL)	Latency, ms (Mean $\pm$ SD)		p value
	Preovulatory	Postovulatory	
I	1.83 ± 0.14	1.83 ± 0.13	0.92
II	2.88 ± 0.18	2.85 ± 0.14	0.38
III	3.84 ± 0.19	3.82 ± 0.16	0.61
IV	5.01 ± 0.21	5.05 ± 0.20	0.15
V	5.81 ± 0.19	5.71 ± 0.18	0.01*
IPL I–III	2.00 ± 0.18	1.99 ± 0.14	0.68
IPL III–V	1.94 ± 0.19	1.89 ± 0.16	0.048*
IPL I–V	3.95 ± 0.18	3.88 ± 0.16	0.017*

On the fifth, seventh and eighth line of Table 3, * denotes statistically significant value ($p < 0.05$)

anovulatory cycle, amenorrhea and women planning to not have conception uses long term estrogen or progesterone or their combination as hormonal replacement; it was found that in many studies there exist change in hearing ability in these females who were under hormonal replacement therapy or oral contraceptives. Hence the aim of the present study was to find out differences in ABR in pre- and postovulatory phases of menstrual cycle in normal menstruating women.

In this study, stimulation of both ears had significant shortening in WL-V and IPLs III–V, I–V occurred in postovulatory phase. Other WLs and IPLs showed generally of lower values in postovulatory phase than in preovulatory phase, a rare finding showing significant differences between pre- and postovulatory ABR (on WL V and IPLs III–V and I–V). Similar increase in the WLs III, V and IPL I–V associated with high estrogen state at the mid cycle phase (12–15 days) i.e. preovulatory phase has been reported [9]. It has also been reported that there lies a tendency of increase in peak latency of all waves and IPLs in mid-cycle and decrease in latencies in mid-luteal with the shortest latencies occurring during menstruation [21]. However, the findings of the present study are contradictory to the findings which presumed influence of estrogen in neuronal plasticity as well as the levels of neurotransmitters resulting in lower values of WLs and IPLs during the estrogen treatment period than at the baseline period [13]. Moreover, WLs and IPLs of shorter values have been shown during the periovular phase with respect to the luteal phase and follicular phase for WL I and IPLs I–V in ABR in premenopausal women before and after taking oral contraceptives [14]. Estrogen treatment in postmenopausal has been shown to be associated with women showed slowing down of hearing loss in aging postmenopausal women [26].

The exact mechanism of action of ovarian hormone in hearing pathway is still not clear. Estrogen has been proposed by some investigators as neuroprotective [21], and vasoprotective, as it is believed to produce favorable effects in axonal sprouting, regenerative responses, enhanced synaptic transmission and enhanced neurogenesis. Further its effects in direct modulation of neurotransmitter receptor functions, and antioxidant activities have been attributed for decrease in latency of ABR in estrogenic phase [27]. However, presently there are emerging findings suggesting that progesterone is neuroprotective by increasing brain derived neurotrophic factor expression and by protecting against glutamate toxicity in a mitogen activated protein kinase and phosphoinositide-3 kinase dependent manner in cerebral cortical explants [28]. Evidence also shows that in females with premature ovarian failure estrogen replacement alone increase the peak latency of wave V and IPL I–V while combination of

estrogen and progesterone decreases the latencies [29]. It has also been hypothesized that estrogen is responsible for electrolyte imbalance and thereby disturbing the inner ear function [30]. Moreover, it is believed to be responsible for brainstem synaptic impairment, presumably by producing ischemic changes [5] that may influence cochlear blood flow and potentially hearing functions [23]. The decreased latency in the postovulatory phase ABR in the present study might be due to the effects of progesterone on hearing pathway. It might be modulating combined hearing pathway of both ears. Progesterone has been thought to be responsible for neuroprotective effects mediated by its reduced metabolites 5 α -dihydroprogesterone and 3 α , 5 α -tetrahydroprogesterone [31], regulating blood flow and electrolyte balance in the inner ear. This has been further supported by the fact that serum progesterone levels correlated were with decreased cerebral edema after traumatic brain injury [32] and on administration of progesterone in acute traumatic brain injured patient (TBI), it helped treating TBI patient. Importance of progesterone for brain cells, may explain why progesterone levels are so high during pregnancy [22]. The same reasons can be attributed for the latency shortening (proper hearing) in postovulatory phase of menstrual cycle in the present study. The major limitations of the present study include lack of estimation of sex hormone profile whereas confirmation of ovulation based on basal body temperature and previous menstrual calendar data.

Conclusions

It can be concluded that hearing conduction as measured by ABR is better in postovulatory phase as compared to preovulatory phase of menstrual cycle. Phases of menstrual cycle affect hearing as measured by ABR due to variation in levels of female sex hormones; progesterone is the likely hormone responsible for the increase in the conduction of auditory pathways in women of reproductive age group. Further the present study suggests, to explore the mechanism of progesterone on auditory pathway.

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Conflict of Interest None.

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