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Original Article

Effect of Lesion of Central Amygdala on Heart Rate Variability in Albino Wistar Rats

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ABSTRACT

Background and Aim: Heart rate variability (HRV) reflects the pre-frontal cortex (PFC) and amygdalar connections. HRV is also influenced by emotions, which is controlled by limbic system. Thus, amygdala-limbic connection is suggested to be the major integrator of HRV and the alterations in the regulation lead to cardiometabolic risks. The present study was conducted to assess the central amygdala in the heart rate variability in albino Wistar rats. **Methods:** A total of 8 albino Wistar rats were taken for the study. Stereotaxic procedure was performed and lesion was made into the central amygdalar nuclei bilaterally. HRV was assessed before and after lesion of central amygdala. **Results:** Following lesion of central amygdala, there was decrease in total power (TP), high frequency (HF), and other time domain indices of HRV, while there was increase in low frequency (LF) and LF-HF ratio. **Conclusion:** Lesion of central amygdala decreased TP indicating the crucial role of central amygdala in the control of HRV. The control of central amygdala on sympathovagal balance is profound.

Key words: Heart rate variability, Lesion, Central amygdalar nucleus, Stereotaxy, Wistar rats.

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INTRODUCTION

Emotion, which is essential for well-being of the individual, has been suggested to be governed by pre-frontal cortex (PFC), anterior cingulate cortex (ACG), and amygdala. Though median amygdala is known to involve in emotion regulation,[1] the role of central amygdala has not been studied yet. Also, the role of amygdala in the regulation of autonomic functions is not fully elucidated. However, amygdala plays a greater role in emotion, rage, and sexual behaviour.

It has been indicated that heart rate variability (HRV) reflects the strength of prefrontal cortex (PFC)-amygdala connections, and it has also been proposed that the baseline HRV is the output of this functional interaction between PFC and amygdala.[2] HRV is the reflector of emotional health of the individual and emotion per se is intricately linked to heart functions including HRV. Thus, amygdala-limbic connection is suggested to be the major integrator of HRV and the alterations in this regulation could be the physiological basis of cardiometabolic risks.[3] However, the role played by central amygdala, which is the core of limbic connections in the brain, are not been properly assessed to date. Therefore, we planned to assess the role of central amygdala, the key component of limbic system on HRV in experimental rat model.

Currently, cardiovascular (CV) risks are more prevalent in society, which is mainly due to the increased levels of stress. Limbic system plays a major role in regulation of stress and emotional reactions of the body. Amygdalar component of limbic-hypothalamic connections is involved in stress modulation. HRV is an established maker of emotional wellbeing of the individual. However, the role of interaction between central amygdala on HRV expression has not been studied yet. Therefore the present study was conducted to assess the bilateral electrolytic lesion of central amygdala nucleus on cardiovascular risk in albino Wistar rats.

MATERIALS AND METHODS

This interventional study was conducted in the Animal Research Laboratory of Physiology department of Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India. The experimental protocol was approved by Postgraduate Research monitoring Committee (PGRMC) JIPMER, Puducherry and the Animal Ethics Committee of Pondicherry University.

Animals

Twenty-four healthy male albino Wistar rats weighing 190 to 250 g, about 3 to 6 months old were procured from the registered vendor (Biogen,

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Bangalore). The rats were housed individually in clear Perspex cages in a paddy husk floor with standard rat chow diet for habituation to the laboratory conditions. Food and water were available ad-libitum in a temperature-controlled environment of 21 ± 1°C with12-h light/dark cycle.

Procedures

Electrolytic nuclear lesion

Stereotaxy was performed following the procedure as adopted by us.[4] It is done under complete anaesthesia following CPCSEA guidelines. Ketamine is used as anesthesia during the procedure. The anaesthetized animal was placed in a stereotaxic apparatus, with the incisor bar set at approximately 5.5 mm below horizontal zero to achieve a flat skull position. An incision was made to expose the rat skull. Electrolytic lesion was made by introducing the electrode at stereotaxic coordinates for central amygdale (anterior = 4.6 mm to lambda, lateral = ±3.4 mm and vertical = 7.4 mm), which was obtained from stereotaxic atlas of rat brain by Konig and Klippel[5] and by allowing the anodal current of 0.5 mA to pass through the electrode for 25s.

Heart Rate Variability

HRV is recorded in rats after anaesthetizing the rats with Ketamine (0.25mg / 250 g of body weight). The rat is completely anaesthetized since it is an invasive procedure. Platinum needle electrodes were inserted and lead II ECG was recorded for 5 min. HRV is recorded in rats using the software Labscribe. Analysis of HRV was done using Kubios 3.0 standard, as prescribed by Task Force of HRV.[6] Frequency domain indices (FDI) such as total power (TP), low frequency component expressed as normalized unit (LFnu), high frequency component expressed as normalized unit (HFnu), ratio of low-frequency power to high-frequency power of heart rate variability (LF-HF ratio) and time domain indices (TDI) such as mean of interval between R to R wave of ECG (mean RR), square root of the mean squared differences of successive normal to normal intervals (RMSSD), the number of interval differences of successive NN intervals greater than 50 ms (NN50) and the proportion derived by dividing NN50 by the total number of NN intervals (pNN50) were recorded.[7] All the HRV parameters were recorded before and after electrolytic lesion of central amygdala nucleus.

Statistical Analysis of Data

All data were given as mean±SD. Pre and post intervention data were analyzed by paired *t* test, and percentage change was calculated. P value <0.05 was considered as statistically significant.

RESULTS

Table 1 shows the comparison of HRV indices following bilateral electrolytic lesion in the central amygdala in albino Wistar rats. Among the frequency domain indices, TP (P=0.012) and HFnu (P=0.025) were significantly decreased and LFnu (P=0.032) and LF-HF ratio (P=0.038) were significantly increased following central amygdala nuclear lesion. All the time domain indices [RMSSD (P=0.022), SDNN (P=0.008) and NN50 (P=0.017)] except mean RR and pNN50 were significantly reduced following bilateral electrolytic lesion in central amygdala in albino Wistar rats.

Figure 1 shows percentage change in TP, LF-HF ratio, SDNN and RMSSD following bilateral electrolytic lesion in the central amygdala in albino Wistar rats. Following bilateral electrolytic lesion of central amygdala, there was a decrease in TP by 47%, SDNN by 39% and RMSSD by 47%, while LF-HF ratio was increased by 87%.

Table 1: Comparison of HRV indices following bilateral electrolytic lesion in the central amygdala in albino Wistar rats (n=8).

Parameters	Before lesion	After lesion	P value
FDI of HRV:			
TP (ms^2)	8151.4±770.6	4306.5±301.60*	0.012
LFnu	54.06±8.35	68.91±7.99*	0.032
HFnu	45.72±6.00	31.02±2.78*	0.025
LF-HF ratio (ms^2)	1.183±0.27	2.22±0.33*	0.038
TDI of HRV:			
Mean RR (ms)	335.18±58.46	374.8±61.65	0.156
RMSSD (ms)	72.71±10.28	43.9±5.62*	0.022
SDNN (ms)	73.33±7.11	38.37±3.63**	0.008
NN50	154.87±10.15	95.28±31.05*	0.017
pNN50 (%)	28.47±2.94	20.13±1.83	0.062

Values are expressed as mean ± SD; Statistical analysis was done by paired *t* test. The P value less than 0.05 was considered statistically significant

*P<0.05, **P<0.01

*represents comparison of the values of lesion with their pre-lesion values

FDI: Frequency domain indices; TP: Total power of HRV; LFnu: Low-frequency power normalized; HFnu: High-frequency power normalized; LF-HF ratio: Ratio of LF to HF; SDNN: Standard deviation of normal to normal (NN) interval; TDI: Time domain indices; RMSSD: Square root of the mean of the sum of the squares of the differences between adjacent NN intervals; SDNN: Standard deviation of NN intervals; NN50: Number of interval differences of successive NN intervals greater than 50; pNN50: NN50 counts divided by all RR intervals.

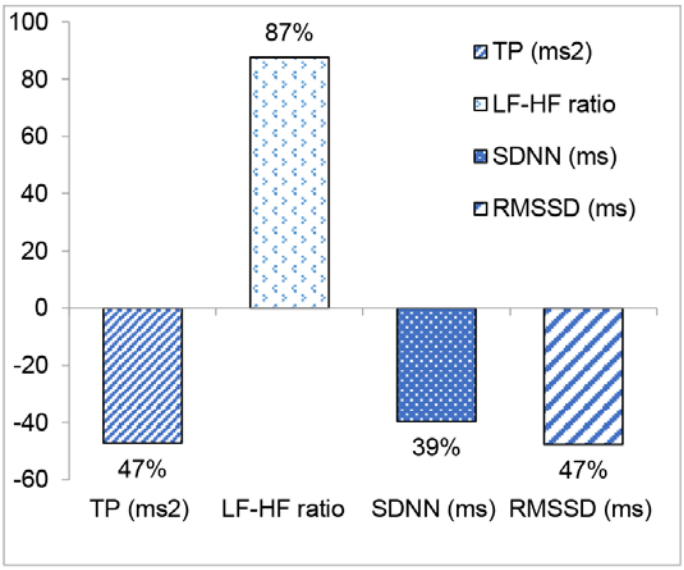

Figure 1: Percentage change in TP, LF-HF ratio, SDNN and RMSSD following bilateral electrolytic lesion in the central amygdala in albino Wistar rats (n=8). TP: Total power of HRV; LF-HF ratio: Ratio of LF to HF; SDNN: Standard deviation of normal to normal (NN) interval; RMSSD: Square root of the mean of the sum of the squares of the differences between adjacent NN intervals.

DISCUSSION

HRV analysis has been established as a marker of CV risk assessment.[8,9] Reduced HRV is reported to a marker of cardiometabolic risk.[10,11] Amygdala has been implicated in the regulation of HRV.[2] Recently we

have reported role of medial amygdala in HRV and cardiometabolic functions.[12] However, the role of central amygdala on HRV has not been studied yet.

TP of HRV represents the quantum of heart rate variability and is also a marker of overall cardiac vagal modulation.[13] There was significant decrease in TP following bilateral electrolytic lesion of central amygdala. Thus, these findings indicate that central amygdala promotes the activity of brain centers that stimulate HRV. All the time domain indices (RMSSD, SDNN, NN50 and pNN50) were significantly decreased following lesion of central amygdala indicating the central amygdala plays an important role in the improvement of vagal drive of HRV. The time domain indices are markers of vagal modulation of cardiac activities. Decreased values of time domain indices represent deteriorating CV health and increased CV risks.

Following lesion of central amygdala, there was decreased TP and time domain indices, all indicating that the central amygdala facilitates HRV, promotes vagal activity and improves CV responses. It has been suggested that influence of amygdala on HRV is mediated through frontal cortex.[2] It has been observed that HRV control of amygdala is linked to regulation of stress and emotion. Higher HRV levels at rest were related to stronger functional coupling between right amygdale and mPFC/ACC. In left vlPFC, a major interaction was observed between HRV and age. In younger than in older adults, stronger functional coupling between vlPFC-amygdala was related to higher HRV levels.[14] Amygdala regulates emotional stress which is having greater connection with HRV. HRV is the marker for cardiovascular risks and its associated risks. Hence amygdale plays a role in maintaining cardiovascular health of an individual. Report indicates that the medial prefrontal cortex (mPFC) and adjacent ACG control.[15] The functions of amygdala during emotion, which is stated to be essential to support well-being of the individual. However, the connection and neural link of amygdala-mPFC circuit in the control of the autonomic functions are less studied. In the present study, we have evaluated the control of HRV by amygdala in younger albino rats. We found that higher levels of HRV were associated with injections of L-arginine into central amygdala in young adult rats. The results of the present study are in agreement with previous report that vl-PFC is involved in control of emotion mainly in younger population.[16-17] Thus, findings of the present study conducted to assess neuro-visceral integration in the experimental rat models indicate that increased HRV could be linked to neural processes that may influence emotional regulation in younger individuals.

Thus, from the reports of present study it appears that central amygdala is involved in regulation of HRV through their autonomic control. The sympathovagal connection to the heart is controlled by a brain structures that include prefrontal cortex, ACG, amygdala, and hypothalamus and parts of the medulla.[18] Central amydala appears to promote HRV. The prefrontal cortex include prelimbic structures involved in neurocognitive and visceral-motor functions.[19] The central amygdala-anterior hypothalamic connection could be an important link for control of viscero-sensory and visceromotor reflexes and regulation of cardiometabolic function, in which nitric oxide plays a crucial neurotransmitter role.

CONCLUSION

TP of HRV was reduced and LF-HF ratio was increased among FDI, and RMSSD, SDNN and NN50 were significantly reduced following bilateral electrolytic lesion. Thus, it appears that central amygdala nuclei are involved in the positive control of HRV. The control of central amygdala on sympathovagal balance could be profound.

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ABBREVIATIONS

HRV: Heart Rate Variability; **PFC:** Pre-Frontal Cortex; **ACG:** Anterior Cingulate Cortex; **CV:** Cardiovascular; **FDI:** Frequency Domain Indices; **TP:** Total Power; **LFnu:** Low Frequency Component Expressed as Normalized Unit; **HFnu:** High Frequency Component Expressed as Normalized Unit; **LF-HF Ratio:** Ratio of Low-Frequency Power to High-Frequency Power of Heart Rate Variability; **TDI:** Time Domain Indices; **SDNN:** The Standard Deviation Of NN Intervals; **RMSSD:** Square Root of The Mean Squared Differences of Successive Normal to Normal Intervals; **NN50:** The Number of Interval Differences of Successive NN Intervals Greater than 50 ms; **pNN50:** The Proportion Derived by Dividing NN50 by the Total Number of NN Intervals.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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