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Original Contribution

HEART RATE VARIABILITY AFTER APPLICATION OF NOCICEPTIN ANALOG N/OFQ(1-13)NH_2 IN SPONTANEOUSLY HYPERTENSIVE RATS

P. Markova[1*], R. Girchev[1], E. Naydenova[2], L. Vezenkov[2]

[1]Department of Physiology, Medical Faculty, Medical University, Sofia
[2]Department of Organic Chemistry, University of Chemical Technology and Metallurgy, Sofia

ABSTRACT

PURPOSE: We investigated the effect of nociceptin analog N/OFQ(1-13)NH_2 on the fast oscillations of inter-pulse interval (IPI) on conscious normotensive Wistar rats and spontaneously hypertensive rats (SHR).
METHODS: After a control period N/OFQ(1-13)NH_2 (100 nmol/kg) was applied intravenously. Blood pressure wave was registered directly through a femoral artery catheter during a control period and after N/OFQ(1-13)NH_2 application. The inter-pulse interval (IPI) was determined in terms of time between two consecutive diastolic minimums of the blood pressure wave. In spectrograms for IPI derived by FFT algorithm, spectral power (P) in the low (LF), mid (MF) and high (HF) frequency bands were studied. Sympatho-vagal balance was determined by the relation P_{MF}/P_{HF}.
RESULTS: Five minutes after N/OFQ(1-13)NH_2 application P_{LF}, P_{MF} and P_{MF}/P_{HF} ratio decreased in Wistar rats during first, second and third 10 min interval ($p<0.05$). In SHR N/OFQ(1-13)NH_2 did not change the spectral characteristics of IPI.
CONCLUSIONS: N/OFQ(1-13)NH_2 inhibits the fast oscillations in IPI mediated mainly by the sympathetic nerve activity in Wistar rats. The lack of changes in SHR may be a result of existing high sympathetic drive to the heart which probably leads to reduced capacity of the heart rate control mechanisms to respond to OP_4 stimulation.

Key words: power spectral analysis, nociceptin analog, SHR.

INTRODUCTION

It has been established that endogenous opiate system plays an important role in mediating cardiovascular responses (1). A lot of experimental data demonstrate that nociceptin, elicits its effects via activation of orphan opioid-like receptors OP_4 (also known as ORL_1), (2, 3). Both nociceptin and OP_4 receptors present in neuronal tissues are involved in the regulation of the cardiovascular system (4). Several structural modifications of nociceptin have been made to yield compounds that mimic the action of the parent peptide in the cardiovascular system. Nociceptin (1-13)NH_2 appears to be the smallest peptide in which the activity of the natural peptide is preserved both after intravenous and intracerebroventricular injection (5-7). Nociceptin (1-17)NH_2 or nociceptin (1-13)NH_2 applied intravenously in anaesthetised or conscious rats produces a transient dose-dependent (0.1-100 nmol/kg) fall in systemic blood pressure accompanied by a reduction in the heart rate (5, 7, 8). In pithed rats it was clearly demonstrated that peripheral cardiovascular effects of nociceptin are mediated through OP_4 receptors (9). It has been established that nociceptin in a concentration-dependent manner inhibits noradrenalin release evoked by chemical or electrical stimulation (9, 10). The modulator action of nociceptin on the peripheral activity of the parasympathetic fibres innervating the heart has also been described (11).

In spontaneously hypertensive rats, it is known that a 1.6 fold increased potency of nociceptin in inhibiting [3H] noradrenalin uptake as compared to normotensive Wistar-Kyoto rats existed, and comparable increased level of cardiac OP_4 mRNA and high affinity binding sites for nociceptin (12) were also established. It was suggested that expression of OP_4 receptors could rise in cardiovascular

*** Correspondence to**: *Petya Markova, Department of Physiology, Medical Faculty, Medical University-Sofia, 1 G. Sofiiski Av., Sofia 1431, Bulgaria; E-mail: dmihov@medfac.acad.bg*

diseases, including hypertension {13). Moreover, the SHR displayed increased overall sympathetic nerve activity (14).

Despite the established facts on the modulatory role of nociceptin and its analogs on the autonomic nervous system, there are no reports addressing the participation of nociceptin in the regulation of fast oscillation of heart rate.

The analysis of heart rate variability in the frequency domain (spectral analysis) has been used to evaluate the autonomic modulation of the heart rate (15). The role of endogenous opiate system in the modulation of the fast oscillation of heart rate in SHR characterized by increased overall sympathetic nerve activity is still not established.

Therefore, the aim of the present study was to investigate, using spectral analysis, the effect of nociceptin analog N/OFQ(1-13)NH_2 in the regulation of heart rate variability in the conscious SHR.

MATERIALS AND METHODS:

Experiments were carried out on male, normotensive Wistar rats (W) and on spontaneously hypertensive rats (SHR) of same age of 12-14 weeks. The study was performed in accordance with the Convention on Animal Protection. The animals were housed under standard condition: constant temperature 22 °C; 12/12h light /dark cycle; free access to standard rat chow and tap water. In the SHR group were included only rats with systolic arterial pressure over 170 mmHg previously measured by non-invasive tile cuff method (*Ugo-Basile*). One day before experiments under general anaesthesia (Nembutal – Sigma, in dose 35 mg/kg b.w., i.p.) femoral artery for a continuous blood pressure measurement and femoral vein for drug application were catheterized. To avoid clotting the catheters were preliminary flushed and after that filled with 20 IU/ml heparin in sterile saline. The catheters were tunnelled subcutaneously and exteriorised at the back of the neck. The experiments were performed on conscious
freely moving animals 24 hours after surgical intervention. Arterial blood pressure wave was registered by Gould Statham transducer P23ID connected to computerized data acquisition system Biopac MP100WS through arterial catheter. The analog to digital converted signal was received and monitored by AcqKnowledge 3.8 software.

Arterial blood pressure was registered during 40 min control period. The nociceptin analog N/OFQ(1-13)NH_2 , prepared by solid-phase synthesis and purified by high performance liquid chromatography, was applied by i.v. bolus injection in dose of 100 nmol/kg dissolved in 100 μl of 0.9 % NaCl. The effects of nociceptin analog were studied five minutes after bolus injection of N/OFQ(1-13)NH_2 for nine consecutive 10 minute long intervals. The values of systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure were determined. Inter-pulse interval (IPI) was determined by peak and rate detectors of the AcqKnowledge 3.8 software in terms of time between two consecutive diastolic minimums of the blood pressure wave, thereafter heart rate was calculated. The obtained raw data of investigated parameter were re-sampled for 10 Hz. The spectrogram for IPI was derived from 512 successive values through virtual instrument developed in graphical programming environment Lab VIEW 3.1.1., by using Fast Fourier Transform algorithm. In the spectrograms spectral power (P) in the low (LF), mid (MF) and high (HF) frequency band typical for rats (20-195; 195-605; 605-3000 mHz, respectively) in $msec^2$ was studied (15). The sympatho-vagal balance was determined by the relation of power of mid to high frequency band $P_{MF}/P_{HF.}$

Statistical analysis was performed by Student's t-test. The results are presented as mean±SEM. Differences at a level $p<0.05$ were considered significant.

RESULTS

In SHR systolic, diastolic and mean arterial blood pressure was significantly higher compared to normotensive rats: SAP was 180.7±2.7 *vs*.131.4±3.5 mmHg; DAP was 112.82±4.43 *vs*. 85.61±3.69 mmHg; MAP was 139.27±4.19 vs. 104.34±3.30 mmHg ($p<0.01$). The heart rate did not differ between normotensive and spontaneously hypertensive rats (326.2±7.8 vs. 334.7±8.9 b.p.m.).

In this experiment we have established the same differences in the fast oscillations of inter-pulse interval between normotensive rats and SHR discussed previously (11). The main differences that were established were decreased P_{LF} in SHR compared to Wistar rats (7.76±0.69 $msec^2$ *vs*. 4.36±0.91 $msec^2$, $p<0.05$), displaced sympatho-vagal balance in SHR (0.54±0.08 vs. 0.75±0.07) as a result of reduced P_{MF} in IPI spectrograms in SHR compared to Wistar rats (0.60±0.09 *vs*. 1.13±0.22 $msec^2$, $p<0.01$).

Five minutes after N/OFQ(1-13)NH_2

administration the SAP, DAP, MAP and heart rate did not change in Wistar rats as well as in SHR. The application of N/OFQ(1-13)NH_2 in Wistar rats led to significant decrease in P_{LF} from 7.76±0.69 msec^2 to 3.04±0.66 msec^2 ($p<0.05$) in the first, to 2.14±0.92 msec^2 ($p<0.05$) in the second and to 2.71±0.99 msec^2 ($p<0.05$) in the third 10 minute interval (**Fig.1**). In the course of the fourth investigated period after application of N/OFQ(1-13)NH_2 the spectral power in the low frequency band returned to its control level. The P_{MF} also decreased after N/OFQ(1-13)NH_2 application to 0.4±0.13 msec^2 ($p<0.05$) in the first investigated interval, to 0.50±0.07 msec^2 ($p<0.05$) in the second and to 0.58±0.08 msec^2 ($p<0.05$) in the third 10 minute interval (**Fig.1**). During the fourth interval after N/OFQ(1-13)NH_2 application the P_{MF} reached its initial value. The P_{HF} did not change as a result of N/OFQ(1-13)NH_2 application: 1.65±0.25 msec^2 in the control period; 1.53±0.35 msec^2 in the first; 1.39±0.40 msec^2 in the second and 1.66±0.5 msec^2 in the third investigated period and till the end of experiment they remained in the same range. The P_{MF}/P_{HF} ratio decreased after N/OFQ(1-13)NH_2 application (***Figure 1***) in the same intervals in which P_{MF} decreased: in the first interval to 0.39±0.03 ($p<0.05$); in the second to 0.35±0.09 ($p<0.05$) and in the third to 0.45±0.07 ($p<0.05$). In the fourth 10-minute interval P_{MF}/P_{HF} ratio returned to its control level.

In SHR N/OFQ(1-13)NH_2 application did not lead to any changes in IPI spectrograms as well as in sympatho-vagal balance in all investigated periods (**Fig.1**). The P_{LF} in the control period was 4.36±0.91 msec^2; in the first 10-minute interval it was 4.27±1.05 msec^2; in the second it was 4.16±0.94 msec^2; in the third it was 4.91±1.30 and the fourth interval it was 4.85±1.18 msec^2. The P_{LF} remained in the same range to the end of the experiments. The mid frequency power in the control period was 0.60±0.09 msec^2 in the first 10 minute interval it was 0.53±0.20 msec^2; in the second it was 0.73±0.24 msec^2; in the third it was 0.79±0.34 msec^2; in the fourth it was 0.61±0.11 msec^2. The P_{MF} remained within the same range until the end of the experiments. The high frequency power in the control period was 1.43±0.17 msec^2; in the first 10-minute interval it was 1.13±0.16 msec^2; in the second it was 1.32±0.50 msec^2; in the third it was 1.23±0.40 msec^2; in the fourth it was 1.28±0.51 msec^2. The P_{HF} remained within the same range to the end of the experiments. The P_{MF}/P_{HF} ratio in the control period was 0.54±0.08; in the first 10-minute interval it was 0.51±0.14; in the second it was 0.59±0.15; in the third it was 0.53±0.16; in the fourth it was 0.55±0.16. The P_{MF}/P_{HF} ratio stayed in the same range till the end of the experiments.

DISCUSSION

In our work we did not establish effect of N/OFQ(1-13)NH_2 on the mean value of heart rate 5 min after its application in normotensive rats as well as in SHR. It has been reported previously that intravenous injection of nociceptin in anaesthetised rats produced transient dose-dependent (0.1-100 nmol/kg) fall in systemic blood pressure accompanied by a strong reduction of heart rate (5, 17). The depressor effect of nociceptin in rat cardiovascular system develops within 30-90 s. Similar changes in blood pressure and heart rate were observed in conscious rats (18, 19). In our experiments we investigated the effect of N/OFQ(1-13)NH_2 on the fast oscillations of heart rate 5 minutes after its application. In this way we exclude the non stationary interval of the heart rate signal, results of bolus application of N/OFQ(1-13)NH_2, inappropriate for spectral analysis. The stimulation of OP_4 receptors by N/OFQ(1-13)NH_2 in Wistar rats produced modulation of sympathetic related fluctuations of IPI. The established decrease of P_{MF} in Wistar rats in our study is in line with previously reported inhibitor action of nociceptin on the cardiac sympathetic nerve activity (17, 20). As a result of the decrease of P_{MF} the ratio P_{MF}/P_{HF} displaced to the vagal mediated part. This result is in accordance with previously supposed mechanism for depressor action of intravenous nociceptin on the cardiovascular function. This action most probably is due to the inhibition of the sympathetic tone with concomitant activation of the parasympathetic outflow (17). The decreased low frequency spectral power (P_{LF}), related to the activity of different humoral factors, after N/OFQ(1-13)NH_2 application may be a result of interaction between N/OFQ(1-13)NH_2 and other factors involved in the regulation of heart rate.

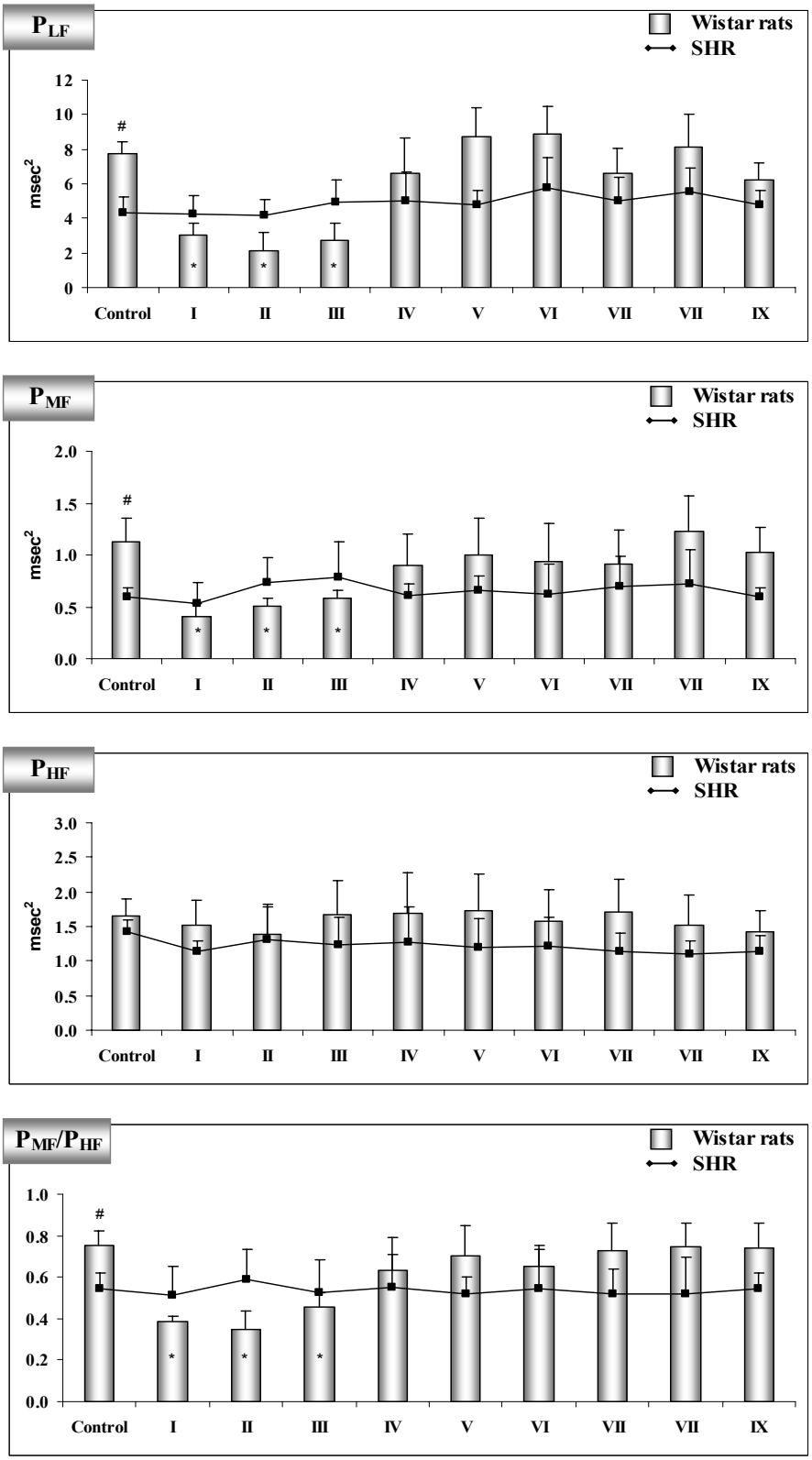

Figure 1: *Spectral power distribution in low (P_{LF}), mid (P_{MF}), high (P_{HF}) frequency band as well as P_{MF}/P_{HF} ratio in normotensive Wistar rats and in spontaneously hypertensive rats SHR during the control period and 5 min. after N/OFQ(1-13)NH_2 in nine consecutive ten minute intervals.*

 # ($p<0.05$) shows significant differences between Wistar rats and SHR
** ($p<0.05$) shows significant effects as a result of intravenous application (100 nmol/kg b.w.) of nociceptin analog N/OFQ(1-13)NH_2 compared to control value*

However, in contrast to Wistar rats the fast oscillations of the heart rate in spontaneously hypertensive rats were not affected after N/OFQ(1-13)NH_2 application. The lack of changes in the P_{MF} in SHR is most probably due to an established increased overall sympathetic nerve activity in spontaneously hypertensive rats. The increased mean level of the sympathetic nerve activity alters the ability of the heart rate to respond to deviations in the sympathetic nerve activity around the mean level (21). The already suppressed P_{MF} in IPI as well as the decreased cardiac sympatho-vagal balance ratio in SHR could not be further affected by N/OFQ(1-13)NH_2.

CONCLUSION

In normotensive Wistar rats nociceptin analog application N/OFQ(1-13)NH_2 led to a decrease in the sympathetically mediated fast oscillation of the heart rate. This effect we observed 5 min after N/OFQ(1-13)NH_2 application during three consecutive 10 min intervals. This result suggests that besides shortly elicited bradycardiac and hypotensive responses, reported previously, intravenously administered N/OFQ(1-13)NH_2 modulate sympathetically mediated fast oscillations in heart rate. In spontaneously hypertensive rats administration of N/OFQ(1-13)NH_2 does not evoke any changes in the fast oscillations of the heart rate. The lack of changes in the fast oscillations in the heart rate in SHR may be a result of existing SHR high sympathetic drive to the heart which probably leads to reduced capacity of the heart rate control mechanisms to respond of OP_4 stimulation.

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