



THE INFLUENCE OF NOCICEPTIN ANALOGUES ON THE MODULATION OF BLOOD PRESSURE OSCILLATION IN SPONTANEOUSLY HYPERTENSIVE RATS

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ABSTRACT

PURPOSE: The aim of our study was to investigate the influence of nociceptin analogues: N/OFQ(1-13)NH₂; [Dab⁹]/OFQ(1-13)-NH₂ and [Dap⁹]/OFQ(1-13)-NH₂ on the blood pressure oscillations in conscious spontaneously hypertensive rats (SHR).

METHODS: The nociceptin analogues were applied intravenously in equal dose -100 nmol/kg b.w. Blood pressure wave was registered directly through a femoral artery catheter during a control period and after application of nociceptin analogues. In spectrograms of systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure, derived by FFT algorithm, spectral power (P) in the low (LF), mid (MF) and high (HF) frequency band were studied.

RESULTS: NOFQ (1-13)-NH₂ do not influence the blood pressure oscillations in SHR. The replacement of lysine with diaminobutyric acid (Dab) in the 9th position provoke a decrease of sympathetic mediated oscillations in blood pressure spectrograms in SHR. The replacement of lysine with diaminopropanoic acid in the 9th position influence both the sympathetic and neurohumoral mediated fluctuations of blood pressure spectrograms in SHR.

CONCLUSIONS: These results indicated functional role of aminoacide placed in 9th position in the structure of nociceptin for realization of its effects on fast oscillations of arterial blood pressure in both normotensive and spontaneously hypertensive rats.

Key words: power spectral analysis, nociceptin analog, SHR

INTRODUCTION

Nociceptin is an endogenous peptide agonist for the orphan receptor opioid receptor-like 1 (ORL₁) is now known as OP₄ (1). Despite the structural similarities of both OP₄, with the opioid receptors and nociceptin, with opioid peptides, nociceptin and its receptor constitute a novel neurotransmitter system distinct from the opioid receptor systems (2, 3). Recently, the interest to structure-activity relationships of nociceptin - OP₄ receptors increased. The different structural modifications of nociceptin have been prepared and analyzed in an attempt to identify the sequence involved in the

activation and in the binding of nociceptin to OP₄ receptor. Results from structure-activity experiments suggest that the entire sequence of NC may not be required for full biological activities. It has been established that Nociceptin (1-13)NH₂ is a smallest peptide in which the activity of natural peptide is preserve (4, 5). The cationic residues Arg^{8,12} and Lys^{9,13} appear to play a functional role for the occupation of OP₄ receptors (6). The OP₄ receptors are present on axon terminals of neurons in the central and peripheral nervous system and that their activation inhibits neurotransmitter release (7). The established wide distribution of OP₄ receptors (8) and their ability to modulate transmitter release suggest the receptor has the potential to modulate a variety of physiological processes.

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The hypertension is typically associated with an elevated sympathetic nerve activity (9, 10). The development of hypertension in spontaneously hypertensive rats (SHR), a frequently used rat genetic model of hypertension, correlated with the increased overall sympathetic nerve activity (11). In hypertensive rats, it has been found higher level of expression of nociceptin precursor mRNA in aorta or culture of visceral smooth muscle cells in comparison to normotensive rats (12).

The aim of our work was to investigate the effects of nociceptin analogs, modulated in nine positions on the fast blood pressure oscillation in spontaneously hypertensive rats. The fast oscillation of blood pressure has received considerable attention, because the patterns of blood pressure variability may provide important information about different factors involved in cardiovascular regulation (13, 14).

MATERIALS AND METHODS

Experiments were carried out on conscious, male, Wistar rats (n=30), and spontaneously hypertensive rats (SHR), (n=30) at the same age 12-14 weeks. The rats included in experiments were divided in six groups each consisting of 10 animals. In the first and second group, consisted of normotensive Wistar rats and SHR respectively, effect of nociceptin analog N/OFQ(1-13)-NH₂ was investigated. The study of participation of nociceptin analog [Dab⁹]/OFQ(1-13)-NH₂ in which lysine in 9th position was replaced with diaminobutyric acid on the blood pressure variability was performed in Wistar rats and SHR in third and fourth group. The effects of [Dap⁹]/OFQ(1-13)-NH₂ in which lysine in 9th position was replaced with diaminopropanoic acid on the blood pressure variability were investigated in Wistar rats and SHR built-in fifth and sixth groups. The experiments were conducted in accordance with guidelines for the care and use of laboratory animals of the ethical commission at the Medical University - Sofia based on the Convention on Animal Protection. The animals were housed under standard condition: constant temperature 22 °C; 12/12 h light /dark cycle; free access to standard rat chow and tap water. In the SHR groups were included only rats with systolic

arterial pressure over 170 mmHg previously measured noninvasively by tile cuff method (Ugo-Basile). One day before experiments under general anesthesia (Nembutal- Sigma) applied, in dose 35 mg/kg b.w., i.p., catheters were inserted in femoral vein for drugs application and in femoral artery for blood pressure measurement. The catheters were tunneled subcutaneously and exteriorized 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 measured directly in femoral artery by blood pressure transducer Gould Statham P23ID, connected to data acquisition system Biopac MP100WS. The nociceptin analogues N/OFQ(1-13)-NH₂; [Dab⁹]/OFQ(1-13)-NH₂ and [Dap⁹]/OFQ(1-13)-NH₂, prepared by solid-phase peptide synthesis and purified by high performance liquid chromatography were applied in equal dose (100 nmol/kg b.w.), intravenously. After a control period the effects of nociceptin analogs were studied five minutes after its bolus injection within nine consecutive 10 minute intervals. In the arterial blood pressure wave the values of systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure were determined for each heart beat by Acknowledge 3.8 software. The obtained raw data of investigated parameters was simultaneously resampled for 10 Hz. The SAP, DAP and MAP spectrograms were derived from 512 successive values in graphical programming environment Lab View 3.1.1, through Fast Fourier Transform (FFT) algorithm. Spectral power in low (P_{LF}: 20-195 mHz); mid (P_{MF}: 195-605 mHz) and high (P_{HF}: 605-3000 mHz) frequency band were studied.

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 statistically significant.

RESULTS

In SHR systolic, diastolic and mean arterial blood pressure was significantly higher compared to normotensive rats in control condition (**Table № 1**). Application of NOFQ (1-13)-NH₂ or modified in nine position analogs did not change mean values of SAP, DAP and MAP in investigated intervals.

Table 1.

Control period	Wistar rats	SHR	Wistar rats	SHR	Wistar rats	SHR
	I group	II group	III group	VI group	V group	VI group
SAPmmHg	131.4±3.4	178.9±3.1###	132.7±4.4	178.7±2.4###	133.15±3.7	179.0±4.3###
DAPmmHg	85.6±3.6	118.9±4.3###	83.4±3.2	119.2±4.2###	84.7±4.3	120.2±3.0###
MAPmmHg	104.3±3.3	139.3±4.2###	101.9±3.1	139.0±3.9###	99.8±4.8	144.4±4.5###

Effects of NOFQ (1-13)-NH₂ , (Fig. 1). The application of NOFQ (1-13)-NH₂ lead to a decrease of P_{LF} and P_{MF} in Wistar rats in SAP, DAP and MAP spectrograms in the first, second and third investigated 10 minute long interval. The fast oscillations in the high frequency band were not affected by NOFQ (1-13)-NH₂ application. The P_{LF} in SAP, DAP and MAP spectrograms decreased from 2.37±0.31, 2.17±0.39 and 2.24±0.35 mmHg² to 1.46±0.34, 1.29±0.24 and 1.42±0.25 mmHg² in the first investigated interval; in the second 10 min long interval to 1.38±0.33, 1.02±0.20, 1.14±0.10 mmHg² (p<0.05) and to 1.55±0.23, 1.31±0.19, 1.42±0.15 mmHg² in the third investigated interval respectively. The P_{MF} was reduced in the spectrograms of SAP by

34.5%, (from 1.21±0.14 to 0.79±0.07 mmHg²), 47.9% (to 0.63±0.23 mmHg²), 43.7% (to 0.68±0.12 mmHg²); in DAP spectrograms by 46.9% (from 1.11±0.13 to 0.59±0.09 mmHg²), 41.6% (to 0.65±0.07 mmHg²), 43.1% (to 0.63±0.09 mmHg²) and in MAP spectrograms by 42.3% (from 1.26±0.13 to 0.73±0.08 mmHg²), 40.4% (to 0.75±0.07 mmHg²), 36.8% (to 0.79±0.09 mmHg²), (p<0.05) during the first, second and third investigated 10 minute long interval. In the course of the fourth investigated period after application of N/OFQ(1-13)NH₂ the spectral power in the low and mid frequency bands returned to their control level. In contrast to normotensive rats in SHR application of NOFQ (1-13)-NH₂ did not change spectral power of SAP, DAP and MAP spectrograms (Fig. 1).

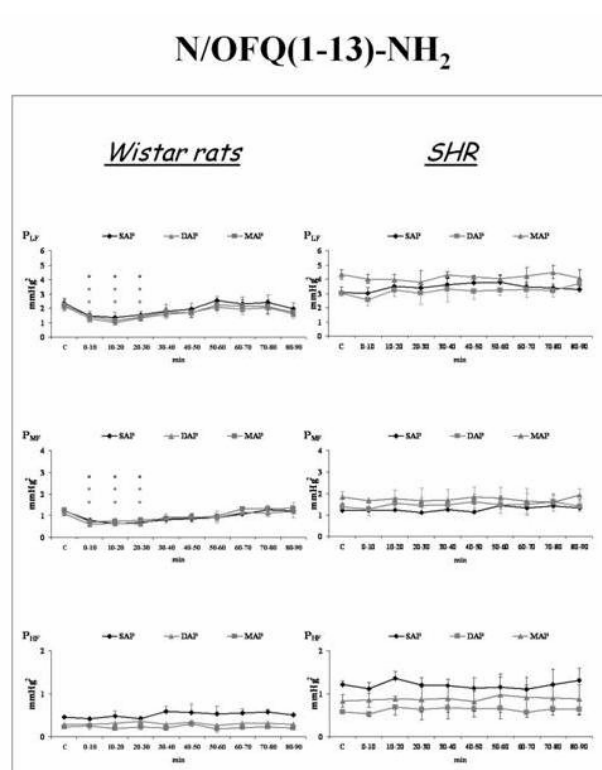


Fig. 1. Effects of N/OFQ(1-13)NH₂ application on spectral power distribution of systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure in low (P_{LF}), mid (P_{MF}), high (P_{HF}) frequency band in normotensive Wistar rats and in spontaneously hypertensive rats (SHR).

* (p<0.05) shows statistically significant effects as a result of intravenously application (100 nmol/kg b.w.) of nociceptin analog N/OFQ(1-13)NH₂ compared to control values.

Effects of $[Dab^9]/OFQ(1-13)-NH_2$, (Fig. 2). The modified in 9th position nociceptin analog $[Dab^9]/OFQ(1-13)-NH_2$ also decreased P_{LF} and P_{MF} in the blood pressure spectrograms in Wistar rats, however decrease of P_{LF} displayed in the second 10 min long interval and prolonged to sixth investigated interval: from $3.10 \pm 0.22 \text{ msec}^2$ in control period to $2.30 \pm 0.28 \text{ msec}^2$ ($p < 0.05$) in the second, to $1.73 \pm 0.29 \text{ msec}^2$ ($p < 0.05$) in the third to $1.65 \pm 0.36 \text{ msec}^2$ ($p < 0.05$) in the fourth, to $1.83 \pm 0.28 \text{ msec}^2$ ($p < 0.05$) in the fifth and to $1.92 \pm 0.50 \text{ msec}^2$ ($p < 0.05$) in the sixth 10 minute interval (Fig.2). In the course of the seventh investigated interval after application of $[Dab^9]N/OFQ(1-13)NH_2$ the spectral power in the low frequency band returned to its control level. The P_{MF} in SAP, DAP and MAP spectrograms was reduced from 2.18 ± 0.30 ; 1.58 ± 0.19 and $1.86 \pm 0.11 \text{ mmHg}^2$ to 1.13 ± 0.30 ; 1.15 ± 0.10 and $1.09 \pm 0.33 \text{ mmHg}^2$ in the third and to 1.08 ± 0.23 ; 0.77 ± 0.14 and to $1.11 \pm 0.27 \text{ mmHg}^2$ in the fourth 10 minute long intervals, respectively ($p < 0.05$). Interestingly,

application of $[Dab^9]N/OFQ(1-13)NH_2$ provoked a decrease in P_{HF} only in the SAP spectrograms in intervals between 10-40 min: from $1.09 \pm 0.11 \text{ mmHg}^2$ in control period to $0.86 \pm 0.06 \text{ mmHg}^2$; $0.72 \pm 0.08 \text{ mmHg}^2$ and $0.79 \pm 0.07 \text{ mmHg}^2$ in the second, third and fourth investigated 10 min long interval respectively, ($p < 0.05$).

In SHR application of $[Dab^9]N/OFQ(1-13)NH_2$ provoked a decrease in P_{MF} in SAP, DAP and MAP spectrograms in the first interval from 1.48 ± 0.09 to $0.87 \pm 0.13 \text{ mmHg}^2$, ($p < 0.05$). In the second interval, P_{MF} in SAP spectrograms returned to its control level $1.43 \pm 0.26 \text{ mmHg}^2$. In DAP and MAP spectrograms the decrease in P_{MF} was preserve and to the second 10 minute long interval. The P_{MF} in the first and second investigated interval was decrease from 1.11 ± 0.24 to 0.58 ± 0.13 and to $0.59 \pm 0.11 \text{ mmHg}^2$, ($p < 0.05$) in DAP spectrograms and from 1.96 ± 0.35 to 0.59 ± 0.08 and to $0.86 \pm 0.1 \text{ mmHg}^2$, ($p < 0.05$) in MAP spectrograms.

$[Dab^9]N/OFQ(1-13)-NH_2$

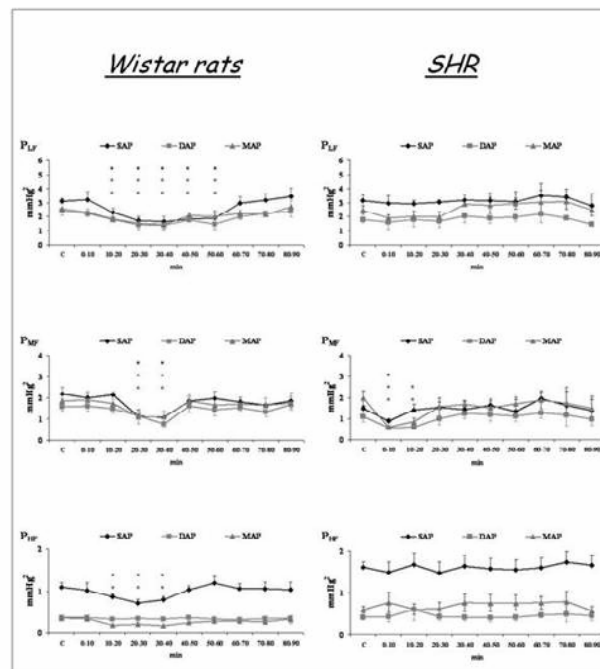


Fig. 2 Effects of $[Dab^9]/OFQ(1-13)-NH_2$ application on spectral power distribution of systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure in low (P_{LF}), mid (P_{MF}), high (P_{HF}) frequency band in normotensive Wistar rats and in spontaneously hypertensive rats (SHR).

* ($p < 0.05$) shows statistically significant effects as a result of intravenously application (100 nmol/kg b.w.) of nociceptin analog $[Dab^9]/OFQ(1-13)-NH_2$ compared to control values.

Effects of [Dap⁹]/OFQ(1-13)-NH₂, (Fig. 3). In Wistar rats application of nociceptin analog [Dap⁹]/OFQ(1-13)-NH₂ in which lysine in 9th position was substitute with diaminopropanoic acid did not change fast oscillations of arterial blood pressure. In contrast to Wistar rats in SHR this analog provoked a decrease of both LF and MF spectral power in the first 10 minute long interval in SAP, DAP and MAP spectrograms. In SAP spectrograms P_{LF} and

P_{MF} decreased from 1.93±0.14 to 0.83±0.10 msec² and from 0.92±0.10 to 0.48±0.06 mmHg²; in DAP spectrograms P_{LF} and P_{MF} decreased from 1.67±0.15 to 0.62±0.14 msec² and from 0.75±0.13 to 0.38±0.05 mmHg², (p<0.05) and in MAP spectrograms P_{LF} and P_{MF} decreased from 2.40±0.26 to 0.89±0.20 and from 0.92±0.04 to 0.39±0.26 mmHg², (p<0.05).

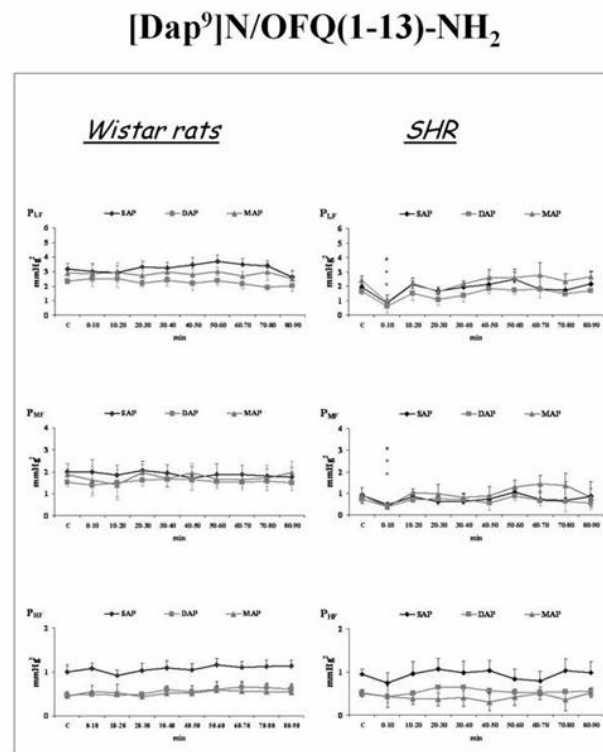


Fig. 3. Effects of [Dap⁹]/OFQ(1-13)-NH₂ application on spectral power distribution of systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure in low (P_{LF}), mid (P_{MF}), high (P_{HF}) frequency band in normotensive Wistar rats and in spontaneously hypertensive rats (SHR).

* (p<0.05) shows statistically significant effects as a result of intravenously application (100 nmol/kg b.w.) of nociceptin analog of [Dap⁹]/OFQ(1-13)-NH₂ compared to control values.

DISCUSSION: In our study we compared the effects of nociceptin analogues: NOFQ (1-13)-NH₂ who is a smallest peptide in which the activity of natural peptide nociceptin is preserve and two analogs in which cationic residue Lys⁹ is replaced with diaminobutyric or diaminopropanoic acid on the fast oscillations of arterial blood pressure in conscious normotensive and spontaneously hypertensive rats. It has been supposed that Lys⁹ plays a functional role for the occupation of OP₄ receptors (6). The differences between lysine, 2,4-diaminobutyric and 2,3-diaminopropanoic acid are expressed in the count of CH₂ groups.

In the normotensive Wistar rats, NOFQ (1-13)-NH₂ application affect the fast oscillations in the low frequency, mediated by neurohumoral factors (14) and in mid frequency mediated mainly by sympathetic nerve activity (11, 13, 14). In normotensive Wistar rats the [Dab⁹]/OFQ(1-13)-NH₂ application, besides of established decrease of low and mid frequency power, provoked by NOFQ(1-13)-NH₂ decreased and high frequency spectral power, mediated by respiration rate (13, 14). The replacement of lysine with diaminobutyric acid in the 9th position, display enhanced effect on the blood pressure variations in Wistar rats.

Interestingly, application of [Dap⁹]/OFQ(1-13)-NH₂ did not affect fast oscillation of arterial blood pressure in normotensive rats. The replacement of Lys⁹ with diaminobutyric acid provoked alterations in all investigated spectral bands, whereas replacement of Lys⁹ with shorter amino acid diaminopropanoic acid did not change variation in arterial blood pressure in Wistar rats. These results indicated functional role of Lys⁹ for realization of effect of nociceptin on the fast oscillations of arterial blood pressure in normotensive Wistar rats. The structural modification related with length of CH₂-chain be able to enhance: [Dab⁹]/OFQ(1-13)-NH₂ or to abolish: [Dap⁹]/OFQ(1-13)-NH₂ the observed effects provoked by NOFQ (1-13)-NH₂. These results indicated that the long of CH₂-chain of cationic residue in nine position have an important role for realization of biological effects of nociceptin analogs on the fast oscillations of arterial blood pressure in Wistar rats.

In contrast to normotensive Wistar rats in SHR, NOFQ (1-13)-NH₂ did not influence fast oscillations of arterial blood pressure in investigated spectral bands. It has been found higher level of expression of nociceptin precursor mRNA in aorta or culture of visceral smooth muscle cells in hypertensive rats compared to those from normotensive rats (12). The established high level of nociceptin precursor mRNA is probably a sign for other compensatory mechanism involved in the regulation of arterial blood pressure in hypertensive states. In maintenance of this supposition vasorelaxant properties of nociceptin in phenylephrine-precontracted segments from cat renal, mesenteric, carotid and femoral arteries with intact endothelium, are established (15).

However, applications of nociceptin analogs in which Lys⁹ is replaced with diaminobutyric or diaminopropanoic acid evoked transient changes of fast oscillations of arterial blood pressure immediately after its application. These results indicated that in SHR structural modification in the nine-position increased ability of nociceptin analogs for achievement of effects on the blood pressure variability. In SHR [Dap⁹]/OFQ(1-13)-NH₂ decreased only sympathetically mediated mid frequency spectral power, whereas effect of nociceptin analog [Dap⁹]/OFQ(1-13)-NH₂ extended and to low frequency fluctuations, generated by many factors including thermoregulation,

activity of bradikinin and renin-angiotensin system (14, 17). The established increased level of overall sympathetic nerve activity (11, 16) in SHR as well as evidences for specific neurohumoral interactions (18) in hypertensive states may explain the differences of responses to application of nociceptin analogs between Wistar rats and SHR.

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