Bulgarian Chemical Communications, Volume 41, Number 2 (pp. 127–132) 2009

Fast oscillations of arterial blood pressure during nociceptin analogues application in *Wistar* rats

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

[1] *Department of Physiology, Medical University, 1 G. Sofiiski Blvd, 1431 Sofia, Bulgaria*
[2] *Department of Organic Chemistry, University of Chemical Technology and Metallurgy, 8 Kliment Ohridski Blvd., 1756 Sofia, Bulgaria*

Received July 16, 2008; Revised September 27, 2008

The effects of nociceptin analogues N/OFQ(1–13)-NH_2 or [Orn^9]/OFQ(1–13)-NH_2 on the blood pressure variability were studied in conscious *Wistar* rats. Arterial blood pressure (ABP) wave was registered directly through a femoral artery catheter by Gould Statham transducer connected to Biopac MP100WS. After a control period the effects of N/OFQ(1–13)-NH_2 or [Orn^9]/OFQ(1–13)-NH_2 applied in equal dose of 100 nmol/kg b.w., *i.v.* were investigated within nine consecutive 10-min intervals. The spectrograms for systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure were derived through Lab View 3.1.1 by Fast Fourier Transform (FFT) algorithm. Spectral power (P) in the low- (LF), mid- (MF) and high- (HF) frequency band in $mmHg^2$ for SAP, DAP and MAP spectrograms were determined. The administration of N/OFQ(1–13)-NH_2 or [Orn^9]/OFQ(1–13)-NH_2 did not change the mean value of ABP during the whole experiment. N/OFQ(1–13)-NH_2 application led to a decrease in P_{LF} in the spectrograms of SAP: from 2.37 ± 0.31 to 1.46 ± 0.34, 1.38 ± 0.33 and 1.55 ± 0.23 $mmHg^2$; DAP: from 2.17 ± 0.39 to 1.29 ± 0.24, 1.01 ± 0.20 and 1.31 ± 0.19 $mmHg^2$ and MAP: from 2.24 ± 0.35 to 1.42 ± 0.25, 1.14 ± 0.10 and 1.42 ± 0.15 $mmHg^2$ in the first three investigated periods, ($p < 0.05$). It also reduced P_{MF} in the spectrograms of SAP by 34.5%, 47.9%, 43.7%; DAP by 46.9%, 41%, 43% and MAP by 42.3%, 44.3%, 36.8%, ($p < 0.05$) in the same investigated intervals. The application of [Orn^9]/OFQ(1–13)-NH_2 did not change the fast oscillation of ABP. The replacement of lysine with ornitine in the 9th position abolished the effects of nociceptin analogue N/OFQ(1–13)-NH_2 on blood pressure variability in *Wistar* rats.

Key words: *Wistar* rats, nociceptin analogues, blood pressure variability.

INTRODUCTION

The autonomic nervous system plays an important role in the regulation of cardiovascular function. Methods to quantify heart rate and arterial pressure variability have emerged as useful tools for evaluating sympathetic and parasympathetic modulation of the cardiovascular system in humans [1] and experimental animals [2]. Blood pressure variability has received considerable attention, not only because enhanced blood pressure variability has been an independent cardiovascular risk factor [3, 4], but also because the patterns of blood pressure variability may provide important information about cardiovascular regulation [5–7].

Nociceptin is the endogenous ligand of a seven-transmembrane domain G protein-coupled receptor referred to as OP_4. Via OP_4 receptor activation nociceptin modulates several biological actions [8]. It has been established that both nociceptin and OP_4 receptors are present in neuronal tissues involved in the regulation of cardiovascular function [9]. An intravenous injection of nociceptin as well as its smallest analogue nociceptin (1–13)NH_2 produced a dose-dependent fall of systemic arterial blood pressure in both anesthetized and conscious rats [10–12]. It has been established that nociceptin inhibits in a concentration-dependent manner noradrenalin release evoked by chemical or electrical stimulation [13, 14]. The modulator action of nociceptin on the peripheral activity of the parasympathetic fibres is also described [15]. Potent and selective ligands are required for investigating the functions regulated by the N/OFQ-OP_4 receptor system in detail and ultimately, for identifying the therapeutic indications of OP_4 receptor agonists and antagonists.

Despite the established facts about the modulator role of nociceptin and its analogues on the autonomic nervous system, there are no reports addressing the participation of nociceptin or its analogues in the regulation of fast oscillation of blood pressure.

The aim of the present study was to determine the effects of nociceptin analogues N/OFQ(1–13)-NH_2 or [Orn^9]/OFQ(1–13)-NH_2 on blood pressure variability in conscious *Wistar* rats.

* To whom all correspondence should be sent:
E-mail: girchev@medfac.acad.bg

EXPERIMENTAL

Synthesis of nociceptin analogues

The solid-phase peptide synthesis by Fmoc (9-fluorenylmethoxycarbonyl) chemistry was used to obtain N/OFQ(1–13)-NH_2 and [Orn^9]N/OFQ(1–13)-NH_2. Rink-amide resin was used as a solid-phase carrier, and 2-(1-OH-benzotriazole-1-yl)-1,1,3,3-tetramethyl-carbamide tetrafluoroborat (TBTU) – as a coupling reagent. The 3-functional amino acids were embedded as follows: Arg-as N^{α}-Fmoc-Arg(Pbf)-OH, Lys-as N^{α}-Fmoc-Lys(Boc)-OH, Orn-as N^{α}-Fmoc Orn(Boc)-OH, Ser-as N^{α}-Fmoc-Ser(tBu)-OH and Thr-as N^{α}-Fmoc-Thr(tBu)-OH. All coupling reactions were performed, at a molar ratio of 3/2.9/3/6/1 for amino acid/TBTU/HOBt/DIEA/resin. The Fmoc-group was deprotected by a 20% piperidine solution in dimethylformamide. The coupling and deprotection reactions were checked by the Kaiser test. The cleavage of the synthesized peptide from the resin was done using a mixture of 95% trifluoroacetic acid (TFA), 2.5% triisopropyl-silan (TIS) and 2.5% water. The protected amino acids were purchased from IrisBiotech (Germany). All other reagents and solvents were analytical or HPLC grade and were supplied by Merck (Germany).

The crude peptides were purified on a reversed-phase high performance liquid chromatography (HPLC) C18 column, using gradient elution with the following solvents: A – H_2O/0.1% TFA and B – CH_3CN/0.1% TFA. The peptide purity was checked by electrospray ionization massspectrometry. The analytical data for the new compound [Orn^9]N/OFQ(1–13)-NH_2 are as follows: tR 7.91 min, > 99% pure, 1368.6 calculated (MH+), 1368.5 observed (MH+).

Experimental design

Experiments were carried out on male, normotensive *Wistar* rats at the age of 12–14 weeks. 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 conditions: 12/12 hours light/dark cycle; 22°C room temperature; free access to tap water and standard rat chow. The effects of nociceptin analogues N/OFQ(1–13)-NH_2 or [Orn^9]/OFQ(1–13)-NH_2 were investigated in two different experimental groups each consisting of 10 animals. For surgical preparation, one day before the experiments the animals were anesthetized with Pentobarbital Sodium (Nembutal, Sigma) 35 mg/kg b.w. given intraperitoneally. The femoral artery for a continuous blood pressure measurement and the femoral vein for drug application were catheterized. To avoid clotting the femoral catheters were flushed with 20 IU/ml heparin in 0.9% sterile saline. The catheters were tunnelled subcutaneously and exteriorized at the back of the neck. Rats were allowed 24 hours to recover from the surgical intervention and the experiments were performed on conscious, freely moving animals. In both experimental groups, blood pressure wave was monitored during 40-min control period, 5-min equilibration and 40-min experimental period. Arterial blood pressure wave was registered by a Gould Statham transducer P23ID connected to computerized data acquisition system Biopac MP100WS through an arterial catheter. The analogue to digital converted signal was received and monitored by AcqKnowledge 3.8 software. The nociceptin analogues N/OFQ(1–13)NH_2 or [Orn^9]/OFQ(1–13)-NH_2 were applied in the first and second experimental groups by *i.v.* bolus injection in a dose of 100 nmol/kg dissolved in 100 µl 0.9% NaCl. The effects were studied five minutes after the bolus injection of nociceptin analogues for nine consecutive 10-min long intervals. The values of systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure were determined by peak and rate detectors of the AcqKnowledge 3.8 software and thereafter the mean values of SAP, DAP and MAP were calculated. The obtained row data of investigated parameter were resampled for 10 Hz. The spectrograms for SAP, DAP and MAP were derived from 512 successive values through a virtual instrument developed in graphical programming environment Lab VIEW 3.1.1., by using Fast Fourier Transform algorithm. In the spectrograms, the 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 $mmHg^2$ was studied [1].

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 AND DISCUSSION

The application of nociceptin analogues N/OFQ(1–13)-NH_2 or [Orn^9]/OFQ(1–13)-NH_2 in a dose of 100 nmol/kg did not provoke changes of the mean values of SAP, DAP and MAP during the whole experimental period in *Wistar* rats (Table. 1).

In the spectral characteristics N/OFQ(1–13)-NH_2 application led to a decrease of P_{LF} in the spectrograms of SAP by 38.2% (from 2.37 ± 0.31 to

1.46 ± 0.34 $mmHg^2$), by 41.7% (to 1.38 ± 0.33 $mmHg^2$), by 34.4% (to1.55 ± 0.23 $mmHg^2$); DAP by 40.4% (from 2.17 ± 0.39 to 1.29 ± 0.24 $mmHg^2$), by 53.1% (to 1.01 ± 0.20 $mmHg^2$), by 39.5% (to 1.31 ± 0.19 $mmHg^2$) and MAP by 36.6% (from 2.24 ± 0.35 to 1.42 ± 0.25 $mmHg^2$), by 49.2% (to1.14 ± 0.10 $mmHg^2$) by 36.9% (to1.42 ± 0.15 $mmHg^2$) in the first three investigated 10-min long periods, ($p < 0.05$), (Fig. 1A). It also reduced P_{MF} in the spectrograms of SAP by 34.5%, (from 1.21 ± 0.14 to 0.79 ± 0.07 $mmHg^2$), 47.9% (to 0.63 ± 0.23 $mmHg^2$), 43.7% (to 0.68 ± 0.12 $mmHg^2$); DAP by 46.9% (from 1.11 ± 0.13 to 0.79 ± 0.07 $mmHg^2$), 41% (to 0.65 ± 0.07 $mmHg^2$), 43% (to 0.63 ± 0.09 $mmHg^2$) and MAP by 42.3% (from 1.26 ± 0.13 to 0.73 ± 0.08 $mmHg^2$), 44.3% (to 0.75 ± 0.07 $mmHg^2$), 36.8% (to 0.75 ± 0.07 $mmHg^2$), ($p < 0.05$) during one and the same investigated periods. In the course of the fourth investigated period after application of N/OFQ(1–13)-NH_2 the spectral power in the low- and mid- frequency bands returned to their control level. The fast oscillations in the high-frequency band were not affected. The application of [Orn^9]/OFQ(1–13)-NH_2 did not change the fast oscillation of arterial blood pressure (Fig. 1B).

The experimental data summarized in the present study demonstrate that intravenous application of N/OFQ(1–13)-NH_2 as well as of [Orn^9]/OFQ(1–13)-NH_2 does not lead to changes in the mean values of arterial blood pressure in conscious *Wistar* rats five minutes after its applications. Previously, it was reported that a transient depressor effect of nociceptin on the cardiovascular system in conscious rats develops within 30–90 s [16]. In our experiments we investigated the effects of N/OFQ(1–13)-NH_2 or [Orn9]/OFQ(1–13)-NH_2 5 minutes after its application. Thus, we excluded the non-stationary interval, caused by bolus injection on blood pressure signal, unsuitable for spectral analysis. In the absence of changes of the mean value of arterial blood pressure in our work we established a reduction in the spectral power in mid- and low-frequency bands as a result of N/OFQ(1–13)-NH_2 application. Mid-frequency blood pressure fluctuations (0.2–0.6 Hz in rats), the so-called Mayer waves, were associated mostly with the sympathetic modulation of vascular tone [17–19]. It has been established that nociceptin inhibits noradrenalin release evoked by chemical or electrical stimulation [20]. The experimental data suggest that nociceptin inhibits transmitter release from sympathetic neurons by a selective blockade of N-type Ca^{2+} channels, which may be of importance for its depressive effect on the cardiovascular system [21]. Evidence has been provided that nociceptin besides neurogenic properties has a direct effect on blood vessels [22]. The experimental data for involvement of prostaglandins and histamine in the effects of nociceptin have been available [23]. It is known that direct vasodilatation produced by nociceptin on the isolated vessels is endothelium independent [24]. Several experimental data have clearly indicated that the action of nociceptin is not involved in the NO-cGMP-dependent pathway [25]. It has been established that muscarinic and alfa-adrenergic receptors are not involved in the vasodilatation evoked by nociceptin in the rat mesenteric vascular bed [24]. The decrease in P_{LF} during N/OFQ(1–13)-NH_2 infusion may be a result of its interaction with a variety of factors associated with LF blood pressure variability (0.02–0.2 Hz in rats) at frequencies below the frequency of the Mayer waves

Table 1. Mean values of systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure in control period and in nine consecutive 10-min long intervals after bolus injection of N/OFQ(1–13)-NH_2 (left panel) or [Orn^9]/OFQ(1–13)-NH_2 (right panel) both applied in a dose of 100 nmol/kg.

	N/OFQ(1–13)-NH_2			[Orn^9]/OFQ(1–13)-NH_2		
	SAP ($mmHg^2$)	DAP ($mmHg^2$)	MAP ($mmHg^2$)	SAP ($mmHg^2$)	DAP ($mmHg^2$)	MAP ($mmHg^2$)
Control	131.40 ± 3.45	85.61 ± 3.69	104.34 ± 3.30	134.02 ± 2.33	86.02 ± 2.62	105.34 ± 2.28
I	130.40 ± 4.91	82.83 ± 4.43	102.10 ± 5.16	137.53 ± 3.59	85.31 ± 5.05	104.76 ± 3.44
II	134.93 ± 4.99	83.83 ± 4.11	104.30 ± 5.14	134.90 ± 3.20	84.62 ± 4.14	105.80 ± 3.99
III	132.73 ± 3.99	84.01 ± 2.64	104.62 ± 4.76	135.93 ± 2.52	86.36 ± 3.17	106.91 ± 3.91
IV	131.80 ± 4.51	84.35 ± 3.45	104.76 ± 4.77	136.56 ± 1.65	88.41 ± 5.56	106.86 ± 3.55
V	132.00 ± 3.31	82.42 ± 4.90	102.14 ± 4.88	137.77 ± 1.22	89.70 ± 4.58	104.06 ± 4.27
VI	133.54 ± 3.12	84.00 ± 3.50	104.56 ± 4.52	137.00 ± 1.31	85.55 ± 5.07	106.00 ± 4.90
VII	130.77 ± 2.93	82.27 ± 4.99	103.22 ± 4.46	133.93 ± 3.60	88.02 ± 5.02	107.06 ± 4.00
VIII	132.62 ± 3.75	83.47 ± 4.58	101.28 ± 4.12	137.42 ± 1.55	83.79 ± 5.71	104.35 ± 4.05
IX	133.01 ± 3.23	85.42 ± 5.27	104.99 ± 4.45	133.13 ± 1.67	82.99 ± 5.21	103.10 ± 3.78

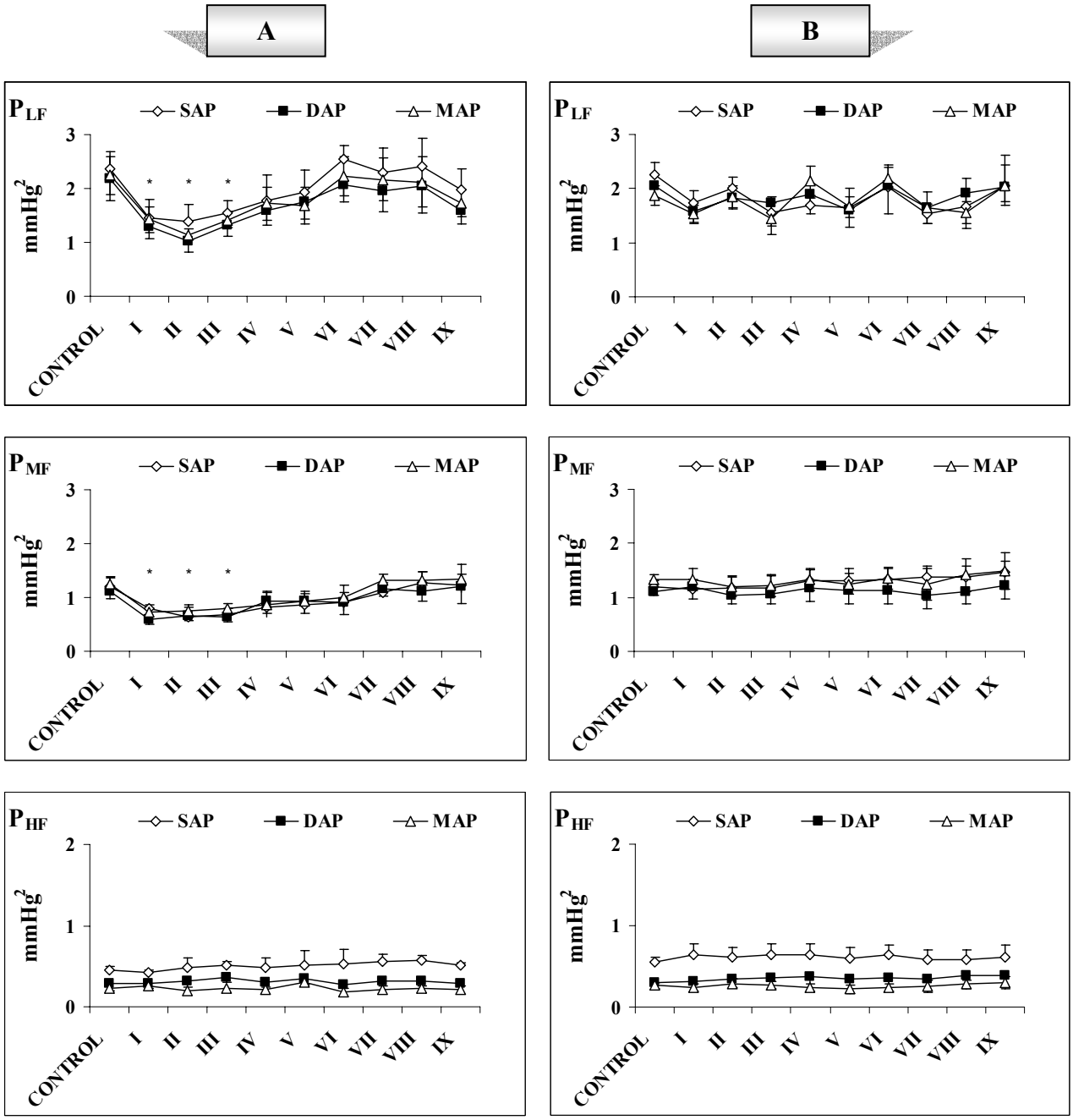

Fig. 1. Power distribution in spectrograms of systolic (SAP), diastolic (DAP) and mean arterial blood pressure (MAP) in low- (P_{LF}), mid- (P_{MF}) and high- (P_{HF}) frequency bands in normotensive *Wistar* rats during the control period and after N/OFQ(1–13)-NH_2 (A) or [Orn^9]/OFQ(1–13)-NH_2 (B), application in a dose of 100 nmol/kg in nine consecutive 10-min intervals. * ($p < 0.05$) shows statistically significant effects as a result of intravenous application (100 nmol/kg b.w.) of nociceptin analogue N/OFQ(1–13)-NH_2 compared to control value.

It is known that spectral power in the low-frequency band is modified by bradykinin [26] and the activity of the renin angiotensin system [26, 27] or catecholamines [28]. The established decrease in P_{LF} and P_{MF} after N/OFQ(1–13)-NH_2 application may be due to its interaction with different factors involved in the modulation of low fluctuations as well as in the direct inhibitor effect on the sympathetic nerve activity. High-frequency (HF) blood pressure variability linked to respiration [1] was not affected neither by N/OFQ(1–13)-NH_2 nor [Orn^9]/OFQ(1–13)-NH_2 applications.

The replacement of lysine with ornitine in the 9th position abolished the effects of nociceptin analog N/OFQ(1–13)-NH_2 on the blood pressure variability in *Wistar* rats.

Acknowledgements*: This work was supported by the National Science Fund through Grant No VU-L-205/2006.*

REFERENCES

1. M. A. Cohen, J. A. Taylor, *J. Physiol.,* **542,** 669 (2002).
2. S. C. Malpas, *Am. J. Physiol.*, **282,** H6 (2002).
3. G. Mancia, A. Frattola, G. Parati, C. Santucciu, L. Ulian, *J. Cardiovasc. Pharmacol.,* **24,** S6 (1994).
4. P. Martinka, J. Fielitz, A. Patzak, V. Regitz-Zagrosek, P. Persson, H. Stauss, *Am. J. Physiol*., **288**, R767 (2005).
5. S. Akselrod, D. Gordon, J. B. Madwed, N. C. Snidman, D. C. Shannon, R. J. Cohen. *Am. J. Physiol.,* **249,** H867 (1985).
6. C. Julien, Z. Q. Zhang, C. Cerutti, C. Barres, *J. Auton. Nerv. Syst.,* **50**, 239 (1995).
7. A. Malliani, M. Pagani, F. Lombardi, S. Cerutti, *Circulation,* **84**, 482 (1991).
8. G. Calò, R. Guerrini, A. Rizzi, S. Salvadori, D. Regoli, *Br. J. Pharmacol*., **129**, 1261 (2000).
9. C. Mollereau, L. Mouledous, *Peptides*., **21**, 907 (2000).
10. C. H. Champion, J. P. Kadowitz, *Life Sci*., **60**, 241 (1997).
11. S. Giuliani, M. Tramontana, A. Lecci, C. A. Maggi, *Eur. J. Pharmacol*., **333**, 177 (1997).
12. R. Bigoni, S. Giuliani, G. Calò, A. Rizzi, R. Guerrini, S. Salvadori, D. Regoli, C. A. Maggi, *Naunyn Schmiedebergs Arch. Pharmacol*., **359**, 160 (1999).
13. B. Bucher, *Naunyn Schmiedebergs Arch. Pharmacol*., **358**, 682 (1998).
14. A. U. Trendelenburg, S. L. Cox, V. Schelb, W. Klebroff, L. Khairallah, K. Starke, *Br. J. Pharmacol*. **130**, 321 (2000).
15. S. Giuliani, C. A. Maggi, *Eur. J. Pharmacol*., **332**, 231 (1997).
16. B. Malinowska, G. Godlewski, E. Schlicker, *J. Physiol. Pharmacol.,* **53**, 301 (2002).
17. C. Julien, S. C. Malpas, H. M. Stauss, *J. Hypertens.,* **19**, 1707 (2001).
18. H. M. Stauss, K. C. Kregel, *Am. J. Physiol.,* **271,** H1416 (1996).
19. H. M. Stauss, P. B. Persson, A. K. Johnson, K. C. Kregel, *Am. J. Physiol*., **273**, H786 (1997).
20. B. Malinowska, J. Piszcz, B. Koneczny, A. Hryniewicz, E. Schlicker, *Naunyn Schmiedebergs Arch. Pharmacol.*, **364**, 233 (2001).
21. K. P. Larsson, U. B. Olsen, A. J. Hansen, *Neurosci. Lett.,* **296,** 121 (2000).
22. M. A. Czapla, H. C. Champion, P. J. Kadowitz, *Peptides*, **18,** 1197 (1997).
23. T. Kimura, K. Kitaichi, K. Hiramatsu, M. Yoshida, Y. Ito, H. Kume, K. Yamaki, R. Suzuki, K. Takagi, *Eur. J. Pharmacol*., **407**, 327 (2000).
24. H. C. Champion, R. L. Pierce, P. J. Kadowitz, *Regul. Pept.,* **78,** 69 (1998).
25. W. M. Armstead, *Brain Res*., **835**, 315 (1999).
26. P. Ponchon, J. L. Elghozi, *Eur. J. Pharmacol.*, **297**, 61 (1996).
27. J. Blanc, G. Lambert, J. L.. Elghozi, *Eur. J. Pharmacol.,* **394**, 311 (2000).
28. A. Radaelli, P. Castiglioni, M. Centola, F. Cesana, G. Balestri, A. Ferrari, M. Di Rienzo, *Am. J. Physiol.*, **290**, H357 (2006).

БЪРЗИ ОСЦИЛАЦИИ НА АРТЕРИАЛНОТО НАЛЯГАНЕ У ПЛЪХОВЕ *Wistar* ПО ВРЕМЕ НА ПРИЛОЖЕНИЕТО НА НОЦИЦЕПТИНОВИ АНАЛОЗИ

Р. А. Гърчев[1]*, П. П. Маркова[1], Е. Д. Найденова[2], Л. Т. Везенков[2]

[1] *Катедра „Физиология", Медицински университет, бул. „Георги Софийски" № 1, 1431 София*
[2] *Катедра „Органична химия", Химикотехнологичен и металургичен университет, бул. „Климент Охридски" № 8, 1756 София*

Постъпила на 16 юли 2008 г.; Преработена на 27 септември 2008 г.

(Резюме)

Ефектите на ноцицептиновите аналози N/OFQ(1–13)-NH_2 и [Orn^9]/OFQ(1–13)-NH_2 върху бързите колебания на артериалното налягане бяха изследвани на будни нормотензивни плъхове *Wistar*. Артериалното кръвно налягане (ABP) беше регистрирано директно през катетър имплантиран във феморалната артерия, чрез трансдюсер за налягане Gould Statham, свързан към Biopac MP100WS. След контролен период ефектите на N/OFQ(1–13)-NH_2 и [Orn^9]/OFQ(1–13)-NH_2 прилагани съответно в еднакви дози 100 nmol/kg т.м., *i.v.* бяха изследвани в 9 последователни 10-минутни интервала. Спектрограмите за систолното (SAP), диастолното (DAP) и средното (MAP) артериално кръвно налягане бяха получени чрез Бърза Фурие трансформация в Lab View 3.1.1. В спектрограмите на SAP, DAP и MAP бяха изследвани спектралните мощности (P) в зоните на ниски (LF), средни (MF), и високи (HF) честоти. Приложението както на N/OFQ(1–13)-NH_2 така и на [Orn^9]/OFQ(1–13)-NH_2 не промени средните стойности на ABP по време на целия експеримент. Приложението на N/OFQ(1–13)-NH_2 предизвика понижаване на P_{LF} в спектрограмите на SAP: от 2.37 ± 0.31 на 1.46 ± 0.34, 1.38 ± 0.33 и на 1.55 ± 0.23 mm Hg2; DAP: от 2.17 ± 0.39 на 1.29 ± 0.24, 1.02 ± 0.20 и на 1.31 ± 0.19 mm Hg2 и MAP: от 2.24 ± 0.35 на 1.42 ± 0.25, 1.14 ± 0.10 и на 1.42 ± 0.15 mm Hg2 в първите три изследвани интервала, ($p < 0.05$). Намалена беше също P_{MF} в спектрограмите на SAP с 34.5%, 47.9%, 43.7%; DAP с 46.9%, 41.6%, 43.1% и MAP с 42.3%, 40.4%, 36.8%, ($p < 0.05$) в същите изследвани периоди. Приложението на [Orn^9]/OFQ(1–13)-NH_2 не предизвика промени в бързите осцилации на артериалното налягане. Заместването на лизин с орнитин в 9^{та} позиция премахва ефекта на ноцицептиновия аналог N/OFQ(1–13)-NH_2 върху вариабилността на артериалното кръвно налягане у плъхове *Wistar*.