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Sympathovagal balance after application of N-modified nociceptin analogues

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The effects of two new N/OFQ(1-13)NH_2 derivates in which the N-terminal Phe was replaced with 1-[(methoxyphosphono) methylamino]cycloalkanecarboxylic acids containing seven AFC7-N/OFQ(1-13)NH_2, (NC7) or eight-membered cycloalkane rings AFC8-N/OFQ(1-13)NH_2, (NC8) on the fast oscillations of the interpulse interval (IPI) as well as on the sympathovagal balance in conscious Wistar rats were investigated. The NC7 or NC8, was applied by i.v. bolus injection in dose 100 nmol/kg b.w. and its effects were studied for nine consecutive 10 minute long intervals. The spectrograms for IPI were derived by using Fast Fourier Transform algorithm. 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) was studied. The sympathovagal balance was determined by the relation of power of mid to high frequency band P_{MF}/P_{HF}. Application of NC7 led to decrease of P_{MF}/P_{HF} from 0.52±0.04 to 0.31±0.08 ($p<0.05$); 0.24±0.06 ($p<0.01$) and to 0.23±0.07, ($p<0.01$) in the first three 10 min long intervals as a result of decrease of mainly sympathetic mediated oscillations of IPI (P_{MF}). Depended by parasympathetic branch of autonomic nervous system fast oscillations of IPI (P_{HF}) were not influenced. Application of NC8 did not affect fast oscillation of IPI as well as sympathovagal balance, but provoked a sustained decrease of heart rate in the course of 70 min, ($p<0.05$). Our results indicated that NC7 led to displacement of sympathovagal balance as a result of decrease of sympathetic determined fast oscillations, whereas NC8 involved a powerful mechanism responsible for long-lasting regulation of heart rate.

Keywords: heart rate variability, conscious rats, N-modified nociceptin analogues

INTRODUCTION

Heart rate variability analysis has been a prevalent technique to assess autonomic influences on the heart. Support for its utility as an index of autonomic tone comes from data which demonstrate that heart rate variability is virtually abolished after sympathetic and parasympathetic blockade [1]. Furthermore, under selected conditions, certain frequencies of heart rate variability are accentuated in response to sympathetic and parasympathetic stimulation [2]. Sympathovagal balance has emerged to describe the dual opposing effects of the sympathetic and parasympathetic nervous systems on the sinus node [3, 4, 5].

The understanding of the role of the N/OFQ - NOP receptor system depends upon the development of selective and highly potent peptide and non-peptide agonist and antagonist ligands [6]. Structure-activity relationship studies identified strategies to render N/OFQ ligands less susceptible to enzymatic degradation. Phosphonopeptides are phosphorus analogues of naturally occurring peptides containing a tetrahedral phosphorus atom. Their importance is obvious from the fact that they are being widely used as enzyme inhibitors and as haptens in catalytic antibody research, because they can be considered as stable mimetics of tetrahedral transition states in ester and amide hydrolysis and formation [7, 8]. It has been established that N-terminal tridecapeptide sequence of nociceptin molecule is sufficient for its full biological activities [9]. The structure-activity relationship studies demonstrated that the N/OFQ sequence can be divided into a N-terminal tetrapeptide "message" critical for receptor activation and a C-terminal "address" important for receptor binding [10]. Recently structure-activity relationships of new N-modified analogues of N/OFQ(1-13)NH_2, with aminophosphonate moiety containing 5-, 7- and 8-membered cycloalkane rings have been reported [11, 12]. In our study we tested the effects of two new N/OFQ(1-13)NH_2 derivates in which the N-terminal Phe was replaced with 1-[(methoxyphosphono) methylamino] cycloalkanecarboxylic acids containing seven AFC7-N/OFQ(1-13)NH_2, (NC7) or eight-membered cycloalkane rings AFC8-N/OFQ(1-13)NH_2, (NC8) on the fast oscillations of the interpulse interval, as well as on the sympathovagal balance in conscious Wistar rats.

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EXPERIMENTAL

Synthesis of nociceptin analogues

The 1-[(dimethoxyphosphono) methylamino] cycloheptanecarboxylic acid and 1-[(dimethoxyphosphono) methylamino] cyclooctanecarboxylic acid were previously prepared using Kabachnik-Fields reaction [13]. The peptides AFC7-N/OFQ(1-13)NH_2 and AFC8-N/OFQ(1-13)NH_2, (Fig. 1) were obtained with good yield by solid phase peptide synthesis - Fmoc (9-fluorenylmethoxy-carbonyl) chemistry, according to the previously described procedure [11, 12]. Rink-amide resin was used as a solid-phase carrier, and 2-(1-OH-benzotriazole-1-yl)1,1,3,3-tetramethyl-carbamide tetrafluoroborate (TBTU) was used as a coupling reagent.

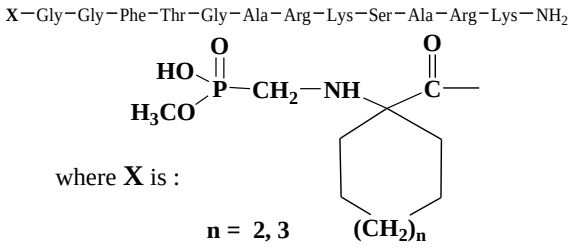

Fig. 1. Sequences of new N-modified analogues of the N/OFQ(1-13)NH_2 with aminophosphonate moiety containing 7- and 8-membered cycloalkane rings

The 3 equivalent of 1-[(dimethoxyphosphono) methylamino]cycloalkanecarboxylic acids were coupled to the growing peptide chain by using TBTU (3 equiv) in the presence of 1-hydroxy benzotriazole (HOBt) dissolved in DMF and in excess of N,N-diisopropylethylamine (DIEA). The coupling reaction time was 15 h. The cleavage of the synthesized peptide from the resin was done using a mixture of 95 % trifluoroacetic acid (TFA), 2.5 % triisopropylsilan (TIS) and 2.5 % water. During the cleavage, one of the methoxy groups from the cyclic aminophosphonic residue was removed. The crude peptides were purified by reversed-phase high-performance liquid chromatography (HPLC) and the molecular weights were determined using electrospray ionization mass-spectrometry.

Experimental design

Experiments were carried out on normotensive Wistar rats (W) at the age of 12-14 weeks, male in sex. The study was performed in accordance with the Convention on Animal Protection. The animals were housed under standard conditions: constant room temperature 22 °C; 12/12 h light /dark cycle; free access to standard rat chow and tap water. The effects of the newly synthesized nociceptin analogues NC7 or NC8 were investigated in two different experimental groups each consisting of 10 animals. Under general anesthesia (Nembutal – Sigma, in dose 35 mg/kg b.w., i.p.) in the femoral artery for a continuous blood pressure measurement and in the femoral vein for drug application catheters were inserted. To avoid clotting, the catheters were previously flushed and after that filled with 20 IU/ml heparin in sterile saline. 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. The arterial blood pressure wave was registered by a 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 wave was registered during a 40 min control period. The N/OFQ(1-13)NH_2 analogue NC7 or NC8, was applied by i.v. bolus injection in dose 100 nmol/kg, dissolved in 200 μl 0.9 % NaCl. The effects of nociceptin analogues were studied five minutes after its application for nine consecutive 10 minute long intervals. In the blood pressure wave the values of inter-pulse interval (IPI) were determined by peak and rate detectors of the AcqKnowledge 3.8 software in terms of time between two consecutive diastolic minima of the blood pressure wave, thereafter heart rate was calculated. The obtained raw data of the investigated parameter were resampled for 10 Hz. The spectrograms for IPI 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 spectrogram 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) was studied [14]. In IPI spectrograms the sympathovagal 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 statistically significant.

RESULTS AND DISCUSSION

Effects of NC7

Application of NC7 did not change the mean values of heart rate. In the control period the heart rate was 342.64±6.11 bpm. In the nine consecutive

10-min long investigated intervals after application of NC7 the mean values of heart rate were: 347.77±5.92 bpm; 347.00±9.95 bpm; 345.42±6.23 bpm; 342.75±4.80 bpm; 344.00±9.22 bpm; 337.17±7.34 bpm; 339.50±8.52 bpm; 344.00±5.20 bpm and 342.84±7.40 bpm, respectively. However, NC7 affected the fast oscillation of IPI spectrograms. Administration of NC7 provoked a decrease in P_{MF} from 1.04±0.13 ms^2 to 0.66±0.16 ms^2, ($p<0.05$) in the first; to 0.55±0.17, ($p<0.01$) in the second and to 0.28±0.05 ms^2, ($p<0.01$) in the third 10 minutes long interval (Fig. 2). In the course of the fourth investigated period after application of NC7 the spectral power in the mid frequency band returned to its control level.

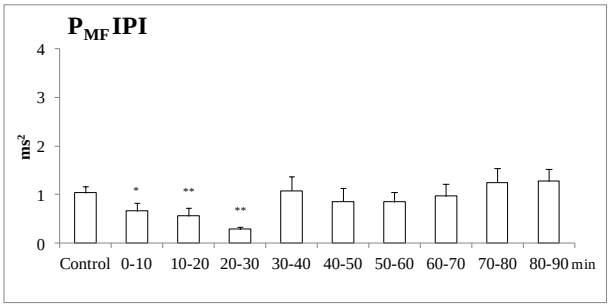

Fig. 2. Mid frequency spectral power (P_{MF}) in interpulse interval (IPI) spectrograms in control period (control) and after application of AFC7-NC(1-13)NH_2, (NC7) in nine consecutive 10 min long intervals

The P_{HF} in the IPI spectrograms did not change as a result of NC7 application: 1.93±0.23 ms^2 in the control period; 1.76±0.29 ms^2 in the first; 2.26±0.38 ms^2 in the second; 1.85±0.27 ms^2 in the third investigated period and till the end of experiment they remained at the same level. The P_{MF}/P_{HF} ratio decreased after NC7 application (Fig. 3) in the same intervals in which P_{MF} decreased: from 0.52±0.04 in the control period to 0.31±0.08 ($p<0.05$) in the first interval to 0.24±0.06 ($p<0.01$) in the second and to 0.23±0.07 ($p<0.01$) in the third investigated interval. In the fourth 10 minute interval the P_{MF}/P_{HF} ratio returned to its control level.

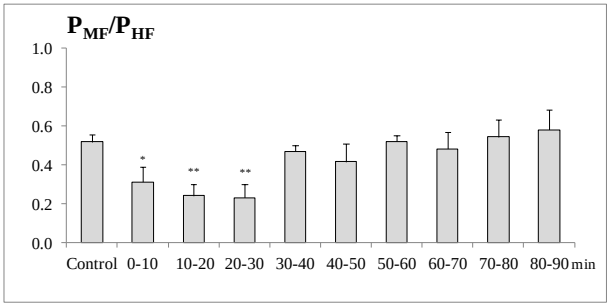

Fig. 3. The sympathovagal balance (P_{MF}/P_{HF}) in the control period (control) and after application of AFC7-NC(1-13)NH_2, (NC7) in nine consecutive 10 min long intervals

The change of sympatovagal balance is a result of decrease of mainly sympathetic mediated oscillations of the IPI spectrograms. Depended by parasympathetic branch of autonomic nervous system fast oscillations of IPI (P_{HF}) were not influenced by application of NC7. Application of NC7 did not affect the power of low frequency oscillations (P_{LF}) in IPI spectrograms neither. The P_{LF} in the control period was 5.21±0.65 ms^2. In the investigated nine consecutive 10-min long intervals during application of NC7 the values of P_{LF} were: 5.57±0.54 ms^2; 5.06±0.64 ms^2; 5.98±0.59 ms^2; 5.87±0.47 ms^2; 5.81±0.69 ms^2; 5.25±0.50 ms^2; 5.15±0.56 ms^2; 5.80±0.57 ms^2 and 5.47±0.64 ms^2, respectively. The mid-frequency oscillations in the interpulse interval are mainly mediated by the sympathetic part of the autonomic nervous system [1, 14]. The power of oscillations in this spectral band, however, can also be modulated by baroreflex mechanisms [14], as well as by interactions of the sympathetic nervous system with different factors affecting the sympathetic nerves activity on a local and on a central level [1, 14, 15]. Our results show a decrease of power in mid frequency oscillations in IPI in the first three investigated intervals. The decrease of the relation P_{MF}/P_{HF}, characterized sympathovagal balance is a result of the decreased mid frequency oscillations in the IPI spectrograms. We suggest that the decreased power of the mid frequency oscillations of the inter-pulse interval is a result of a specific, short-term influence of the sympathetic nerve activity to the sinus node.

Effects of NC8

Differently from NC7, the application of NC8 led to a decrease in the mean values of heart rate in the intervals between 0-70 min (Fig. 4).

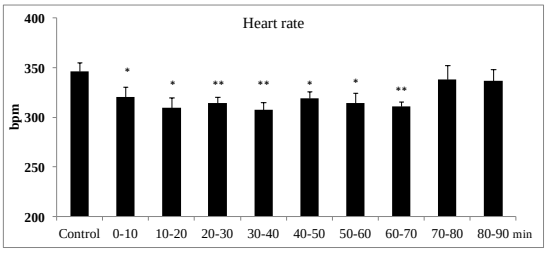

Fig. 4. Mean values of the heart rate in the control period (control) and after application of AFC8-NC(1-13)NH_2, (NC8) in nine consecutive 10 min long intervals

In the control period the heart rate was 346.5±8.7 bpm. As a result of NC8 application the heart rate decreased to 320.5±10.1 bpm, ($p<0.05$) in the interval 0-10 min; to 309.5±10.5 bpm, ($p<0.05$) in the interval 10-20 min; to 314.1±6.5

bpm, ($p<0.01$) in the interval 20-30 min; to 307.3±7.5 bpm, ($p<0.01$) in the interval 30-40 min; to 319.2±6.7 bpm, ($p<0.05$) in the interval 40-50 min to 314.2±10.4 bpm, ($p<0.05$) in the interval 50-60 min and to 311.2±4.8 bpm, ($p<0.01$) in the interval 60-70 min. In the last two intervals 70-80 min and 80-90 min the mean values of the heart rate were restored to the control level: 338.00±14.0 and 336±11.9 bpm, respectively. Interestingly, in difference to NC7, the application of NC8 did not change the spectral characteristics of IPI and sympathovagal balance (Tabl. 1).

Table 1. Spectral power distribution in interpulse interval (IPI) spectrograms in the low (P_{LF}), mid (P_{MF}), and high (P_{HF}) frequency band as well as sympathovagal balance (P_{MF}/P_{HF}) in the control period (control) and after application of AFC8-NC(1-13)NH_2, (NC7) in nine consecutive 10 min long intervals

Investi-gated periods	P_{LF} (ms^2)	P_{MF} (ms^2)	P_{HF} (ms^2)	P_{MF}/P_{HF}
Control	5.60±0.76	1.18±0.29	2.13±0.11	0.53±0.09
0-10 min	5.93±0.22	1.09±0.25	2.66±0.26	0.50±0.12
10-20 min	5.81±0.67	1.04±0.09	2.71±0.42	0.56±0.10
20-30 min	5.52±0.62	0.97±0.08	2.38±0.26	0.53±0.06
30-40 min	5.43±0.83	1.06±0.09	2.27±0.39	0.52±0.08
40-50 min	6.02±0.88	1.30±0.19	2.44±0.34	0.61±0.08
50-60 min	5.71±0.79	1.00±0.12	1.95±0.37	0.53±0.09
60-70 min	5.79±0.52	1.21±0.34	2.43±0.38	0.59±0.08
70-80 min	6.08±0.28	1.08±0.28	2.00±0.23	0.53±0.07
80-90 min	6.05±0.58	1.43±0.21	2.79±0.35	0.58±0.06

In our previous investigations we have established that N/OFQ(1-13)NH_2, the smallest peptide in which the activity of the natural peptide is preserved, participated in the regulation of sympathovagal balance in normotensive Wistar rats [16]. Briefly, the application of N/OFQ(1-13)NH_2 led to a decrease in P_{MF} in the first three investigated intervals, did not affect the power of the high frequency oscillations and decreased the relation of P_{MF}/P_{HF} in the same intervals in which P_{MF} decreased. In our current study we established identical effects provoked by NC7 application on the fast oscillation in IPI. Application of NC7 provoked a decrease in P_{MF}, as well as a decrease in the P_{MF}/P_{HF} ratio in the first three 10 min long intervals. Both N/OFQ(1-13)NH_2 and NC7 did not change P_{HF}. On the other hand, application of NC8 did not lead to changes in the fast oscillation of IPI, as well as in the sympathovagal balance, but provoked a sustained decrease of heart rate in Wistar rats. Such an effect was not observed neither after the application of NC7, nor after applying N/OFQ(1-13)NH_2. The replacement of Phe in position 1 of N/OFQ(1-13)NH_2 with 1-[(methoxyphosphono)methylamino] cycloheptanecarboxylic acid (NC7) did not provoke effects different from that established after application of N/OFQ(1-13)NH_2. In our experiments we established that the replacement of Phe1 of N/OFQ(1-13)NH_2 with 1-[(methoxyphosphono)methylamino] cyclooctanecarboxylic acid (NC8) abolished the action of N/OFQ(1-13)NH_2 on the fast oscillation of the interpulse interval. However, the NC8 administration provoked a decrease in the mean values of the heart rate. Despite of lack of effects of NC8 on the fast oscillation of the interpulse interval, NC8 involved a powerful mechanism responsible for long-lasting regulation of heart rate.

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СИМПАТИКО-ВАГУСОВ БАЛАНС СЛЕД ПРИЛОЖЕНИЕ НА N-МОДИФИЦИРАНИ НОЦИЦЕПТИНОВИ АНАЛОЗИ

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(Резюме)

Изследвани са ефектите на два новосинтезирани N/OFQ(1-13)NH_2 аналози в които N-крайната аминокиселина (фенилаланин) е заместена с 1-[(метоксифосфоно)метиламино]циклоалканкарбоксилна киселина, съдържаща седем AFC7-N/OFQ(1-13)NH2, (NC7) или осем членен циклоалканов пръстен AFC8-N/OFQ(1-13)NH2, върху бързите колебания на интерпулсовия интервал (IPI) и върху симпатико-вагусовия баланс у будни нормотензивни плъхове Wistar. Ефектите на NC7 или на NC8, приложени i.v., бяха изследвани в 9 последователни 10 минутни интервала. Спектрограмите на IPI бяха получени чрез бърза Фурие трансформация. Бяха изследвани мощностите на колебания (P) в зоните на ниски (LF), средни (MF) и високи (HF) честоти, характерни за плъхове (20-195; 195-605; 605-3000 mHz). Симпатико-вагусовият баланс беше определен чрез отношението P_{MF}/P_{HF}. Приложението на NC7 предизвика понижение на стойността на отношението P_{MF}/P_{HF} от 0.52±0.04 на 0.31±0.08 ($p<0.05$); 0.24±0.06 ($p<0.01$) и на 0.23±0.07, ($p<0.01$) в първите три 10 минутни интервала, в резултат на понижението на предимно симпатиково медиираните осцилации P_{MF} на IPI. Мощността на опосредстваните от парасимпатикуса колебания P_{HF} на IPI не бяха повлияни от приложението на NC7. Приложението на NC8 не засегна бързите колебания на IPI и симпатико-вагусовия баланс, обаче предизвика трайно понижаване на сърдечната честота в продължение на 70 минути, ($p<0.05$). Нашите резултати показват, че NC7 предизвиква изместване на симпатико-вагусовия баланс в резултат на понижаването на мощността на симпатиково медиираните осцилации, докато NC8 въвлича механизми, отговорни за дълготрайната регулация на сърдечната честота.