


His-Leu, an angiotensin I-derived peptide, does not affect haemodynamics in rats

Journal of the Renin-Angiotensin-Aldosterone System
October-December 2018: 1–5
© The Author(s) 2018
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1470320318808879
journals.sagepub.com/home/jra


Adrian Drapała¹, Klaudia Bielińska¹, Piotr Konopelski¹,
Leszek Pączek² and Marcin Ufnal¹ 

Abstract

Introduction: The dipeptide histidine-leucine (His-Leu) is formed in the process of converting angiotensin I into angiotensin II. Several studies show that short peptides containing His-Leu may produce significant haemodynamic effects; however, to the best of our knowledge, data on haemodynamic effects of His-Leu are not available in medical databases.

Materials and methods: We evaluated acute haemodynamic effects of intravenous administration of either 0.9% NaCl (vehicle) or His-Leu at a dose of 3–15 mg/kg body weight in anaesthetized 15–16-week-old, male, normotensive Wistar Kyoto and spontaneously hypertensive rats. Chronic effects of treatment with either the vehicle or His-Leu at a dose of 15 mg/kg body weight given subcutaneously daily were determined during continuous telemetry recordings in freely moving rats.

Results: In anaesthetized rats both the vehicle and His-Leu produced a mild and transient increase in blood pressure and no change in plasma renin activity. There was no significant difference in haemodynamics between the rats infused with the vehicle and the rats infused with His-Leu. In chronic studies, seven-day treatment with vehicle and with His-Leu did not affect arterial blood pressure in freely moving rats.

Conclusion: His-Leu does not produce either acute or chronic changes in arterial blood pressure in normotensive and hypertensive rats.

Keywords

Blood pressure, dipeptides, renin–angiotensin system, angiotensin, histidine, leucine

Date received: 16 February 2014; accepted: 12 September 2018

Introduction

The renin–angiotensin system (RAS) consists of a large number of enzymes and angiotensinogen-derived peptides, which exert a wide spectrum of biological actions.^{1–4}

The dipeptide histidine-leucine (His-Leu) is formed in the process of converting angiotensin I into angiotensin II, a key peptide in arterial blood pressure regulation. Strikingly, to the best of our knowledge, haemodynamic effects of this dipeptide have not been evaluated yet, or data are not available in medical databases. In this regard, some dipeptides, such as kyotorphin (L-tyrosyl-L-arginine), an opioid-like analgesic,⁵ and carnosine (beta-alanyl-L-histidine), an antioxidant and metal ion chelating compound,⁶ are known to have strong physiological effects. Moreover, several studies show that short peptides containing His-Leu exert significant haemodynamic effects. For example, a tetrapeptide, alanine-histidine-leucine-leucine (Ala-His-Leu-Leu), and a tripeptide, His-His-Leu, were found to lower arterial blood pressure.^{7–9}

Therefore, in this study we evaluated the effects of His-Leu on arterial blood pressure and heart rate in normotensive rats and spontaneously hypertensive rats which are characterized by increased activity of the RAS.¹⁰

¹Department of Experimental Physiology and Pathophysiology, Laboratory of the Centre for Preclinical Research, Medical University of Warsaw, Poland

²Department of Immunology, Transplantation and Internal Medicine, Medical University of Warsaw, Poland

Corresponding authors:

Marcin Ufnal, Department of Experimental Physiology and Pathophysiology, Medical University of Warsaw, Banacha 1B, Warsaw, 02-097, Poland.
Email: mufnal@wum.edu.pl

Leszek Pączek, Department of Immunology, Transplantation and Internal Diseases, Medical University of Warsaw, Nowogrodzka 59, 02-006 Warsaw, Poland.
Email: lpaczek@wum.edu.pl



Methods

The experiments were carried out according to Directive 2010/63/EU and approved by the Local Ethical Committee. The study was performed on 15–16-week-old, male, normotensive Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats provided by the Central Laboratory of Experimental Animals, Centre for Preclinical Research and Technology, Warsaw, Poland. The animals were fed with standard laboratory chow and maintained on tap water ad libitum.

Acute studies

Rats were anaesthetized with urethane (Sigma-Aldrich, Poland) at a dose of 1.5 g/kg body weight (BW) intraperitoneally. Femoral artery and femoral vein were cannulated with polyurethane catheters. The arterial catheter was connected to the Biopac MP 150 (Biopac Systems, Goleta, USA) for haemodynamic recordings. The venous catheter was used for administration of investigated compounds. The measurements were started one hour after the induction of general anaesthesia. Haemodynamics were recorded 20 min at baseline (before treatment) and 30 min after intravenous infusions. WKY were infused with either 0.5 ml 0.9% NaCl/30 s (vehicle, $n=6$) or His-Leu at a dose of 3 ($n=5$) or 15 mg/kg BW ($n=6$). SHR were treated with either the vehicle ($n=6$) or His-Leu at a dose of 3 ($n=6$), 6 ($n=5$) or 15 mg/kg BW ($n=6$).

Chronic studies

WKY ($n=12$) and SHR ($n=12$) were implanted with a telemetry transmitter (HD-S10, Data Sciences International, St. Paul, USA) under general anaesthesia with ketamine (Bioketan 100 mg/ml, VetoquinolBiowet, Poland) 100 mg/kg BW and xylazine (20 mg/ml, Xylapan, VetoquinolBiowet, Poland) 10 mg/kg BW intraperitoneally, as previously described.¹¹ After seven days of post-surgical recovery, continuous recordings of heart rate (HR) and mean arterial blood pressure (MABP) were started with ART software (Data Sciences International, St. Paul, USA). MABP was calculated as the area under the pressure curve of cardiac cycles recorded over 24 h. The recordings were performed for 10 days: two days of baseline, seven days of treatment and one day of post-treatment period. WKY were treated with either 1 ml 0.9% NaCl (vehicle, $n=6$) or His-Leu at a dose of 15 mg/kg BW, subcutaneously (s.c.), daily ($n=6$). Similarly, SHR were treated with either the vehicle or His-Leu at a dose of 15 mg/kg body weight, s.c., daily.

Plasma renin activity

Anaesthetized WKY were implanted with polyurethane catheters inserted into the left femoral vein (for intravenous

Table 1. Mean arterial blood pressure (MABP, mmHg) and heart rate (HR, beats/min) at baseline in normotensive Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR).

Groups	MABP	HR
Acute studies (anaesthetized rats)		
WKY		
Vehicle	78.0 ± 4.0	294 ± 17
3 mg His-Leu/kg body weight	78.0 ± 3.3	279 ± 6
15 mg His-Leu/kg body weight	82.2 ± 2.3	299 ± 16
SHR		
Vehicle	84.4 ± 1.5	253 ± 5
3 mg His-Leu/kg body weight	88.1 ± 2.2	274 ± 8
6 mg His-Leu/kg body weight	83.9 ± 3.5	245 ± 14
15 mg His-Leu/kg body weight	83.3 ± 1.3	255 ± 8
Chronic studies (freely moving rats)		
WKY		
Vehicle	101.4 ± 2.8	344 ± 7
15 mg His-Leu/kg body weight	104.1 ± 1.7	340 ± 6
SHR		
Vehicle	163.5 ± 3.3	326 ± 4
15 mg His-Leu/kg body weight	164.4 ± 1.7	325 ± 4

administration) and inferior vena cava via the right femoral vein (for blood sampling). Blood samples were collected at baseline and 60 min after the intravenous administration of the vehicle ($n=5$) and His-Leu at a dose of 15 mg/kg BW ($n=7$). A Renin Assay Kit (cat. no. MAK157-1KT, Sigma-Aldrich, Poland) was used to evaluate plasma renin activity. All procedures were performed according to the technical bulletin provided by the producer. The plate was incubated at 37°C for 60 min taking measurements ($\lambda_{ex} = 540/\lambda_{em} = 590$ nm) every 5 min. All experiments were performed in duplicate.

Statistics

Data are expressed as means ± SEM. For the evaluation of MABP and HR responses to treatment, the average over 5 min (acute studies) or 24 h (chronic studies) baseline was compared with the average over 5 min (acute studies) or 24 h (chronic studies) after the treatment, by means of one-way analysis of variance (ANOVA) for repeated measures. Differences between the groups were evaluated by multivariate ANOVA. If ANOVA showed a significant difference, the post-hoc Tukey's test was performed. The Kolmogorov–Smirnov test was used to test normality of the distribution. A value of two-sided $p < 0.05$ was considered significant. Analyses were conducted using Dell Statistica, version 13 (Dell Inc., Tulsa, USA).

Results

Baseline haemodynamic parameters are presented in Table 1.

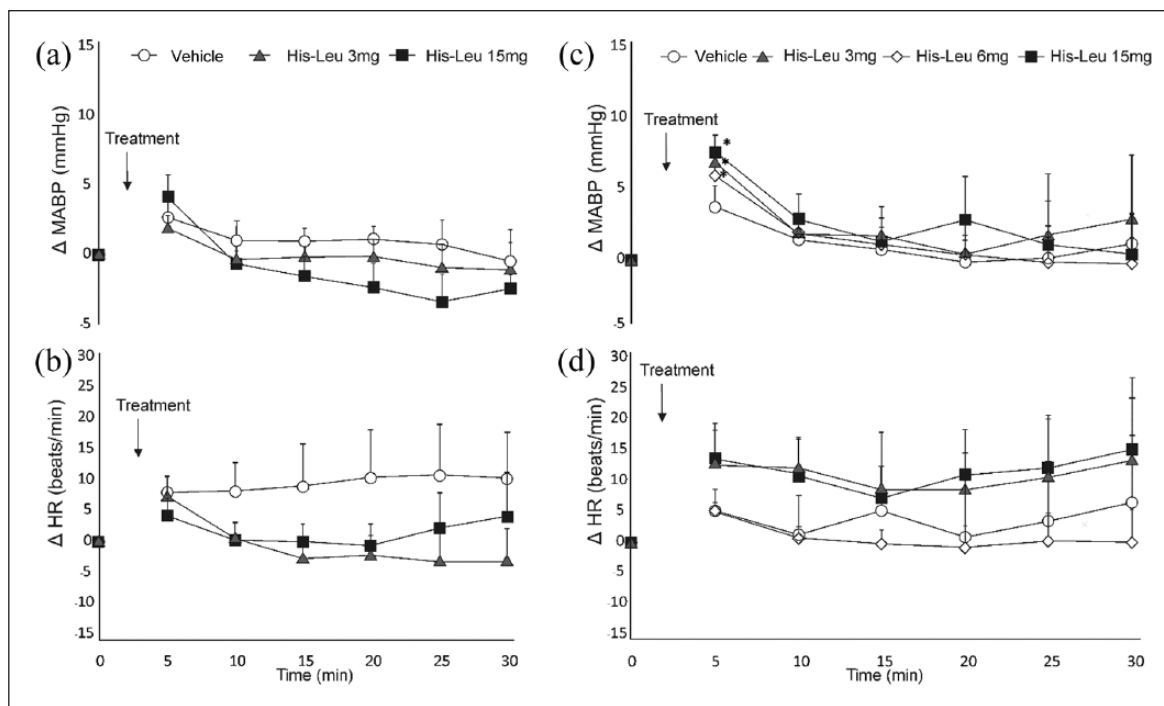


Figure 1. Changes in mean arterial blood pressure (Δ MABP (mmHg)) and heart rate (Δ HR (beats/min)) after the intravenous infusion of either His-Leu (3, 6 or 15 mg/kg body weight) or 0.9% NaCl (vehicle) in anaesthetized normotensive Wistar Kyoto rats ((a) and (b)) and spontaneously hypertensive rats ((c) and (d)). * $p < 0.05$ vs. baseline.

Acute studies

WKY. There was a transient, insignificant increase in MABP and HR after intravenous administration of the vehicle and His-Leu at a dose of 3 and 15 mg/kg BW. There was no significant difference in MABP and HR changes between the groups (Figure 1(a) and (b)).

SHR. There was an insignificant increase in MABP and HR after the treatment with the vehicle and His-Leu at a dose of 3 mg/kg BW. The treatment with His-Leu at a dose of 6 mg/kg BW and 15 mg/kg BW produced a significant increase in MABP for the first 5 min after the infusion ($p < 0.05$). However, the between-group analysis showed no significant difference between the treatment groups (Figure 1(c) and (d)).

Chronic studies

WKY. There was no significant change in MABP and HR in response to treatment with either the vehicle or His-Leu. There was also no significant difference in MABP and HR changes between the two groups (Figure 2(a) and (b)).

SHR. There was a significant decrease in MABP on the sixth and seventh days of treatment with the vehicle. There was also a decrease in MABP, however, not significant, on

the sixth and seventh days in the group treated with His-Leu. The between-group analysis showed no significant differences in MABP changes (Figure 2(c)).

There was a transient, significant increase in HR in rats treated with His-Leu (Figure 2(d)). There was also an increase in HR, however, not significant, in the group treated with the vehicle. The between-group analysis showed no significant differences in HR changes.

Plasma renin activity

There was no significant change in plasma renin activity after the administration of the vehicle (2554 ± 271 vs. 2674 ± 353 Relative Fluorescence Units (RFU), baseline and 60 min after the administration, respectively) or His-Leu (2258 ± 228 and 2448 ± 247 RFU).

Discussion

Our study shows that His-Leu, a dipeptide formed in the process of converting angiotensin I into angiotensin II, does not affect significantly haemodynamic parameters in normotensive and hypertensive rats.

Research continues to broaden our knowledge about angiotensinogen-derived peptides, providing evidence for a key role of the RAS in mammals' homeostasis. His-Leu is a dipeptide formed in the process of synthesis of angiotensin

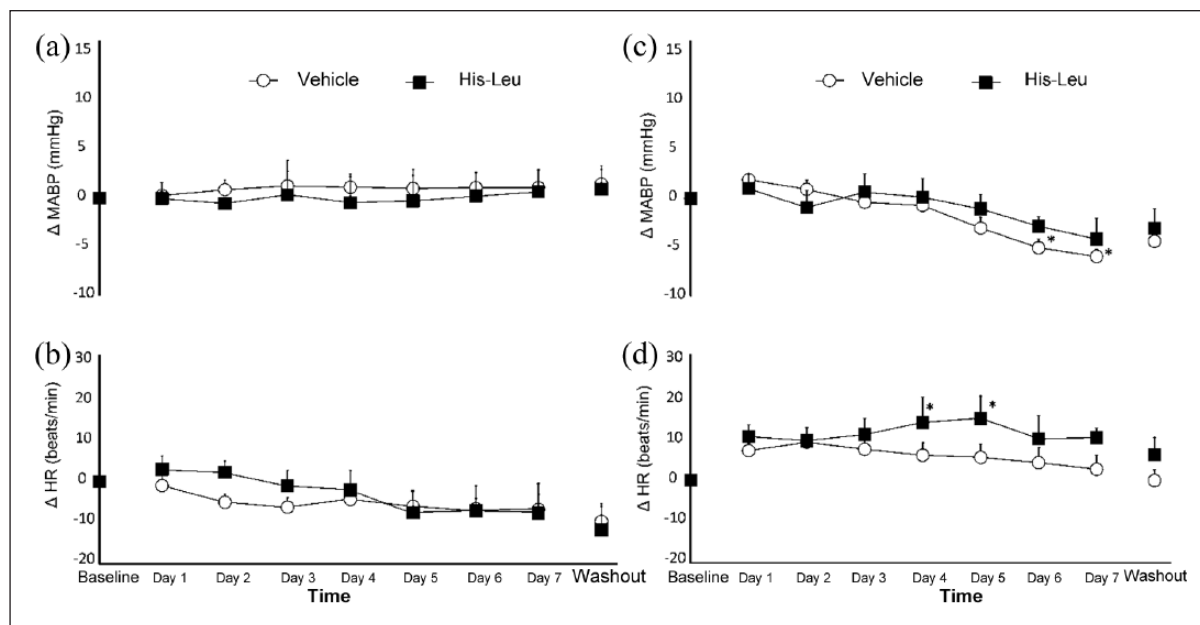


Figure 2. Changes in mean arterial blood pressure (Δ MABP (mmHg)) and heart rate (Δ HR (beats/min)) in freely moving normotensive Wistar Kyoto rats ((a) and (b)) and spontaneously hypertensive rats ((c) and (d)). Baseline: before treatment. Days 1–7: treatment with either 0.9 NaCl (vehicle) or His-Leu at a dose of 15 mg/kg body weight given subcutaneously daily. * $p < 0.05$ vs. baseline.

II, a pivotal player in the control of arterial blood pressure. Some studies suggest that short peptides containing His-Leu sequence lower arterial blood pressure. For instance, a tripeptide, His-His-Leu, derived from soybean paste, was found to exert a hypotensive effect by inhibiting angiotensin-converting enzyme.⁹ In addition, Li et al. showed that tetrapeptide Ala-His-Leu-Leu decreases systolic blood pressure by the same mechanism.^{7,8} However, to the best of our knowledge, haemodynamic effects of His-Leu have not yet been described.

Here, we found that His-Leu did not affect significantly the haemodynamic parameters in normotensive and hypertensive rats. Although we found some mild changes in haemodynamic parameters over the course of acute and chronic experiments, the changes were comparable between the rats treated with the vehicle and with His-Leu. Therefore, it seems that they reflected haemodynamic responses to experimental conditions, such as an increase in blood volume after the treatment.

It is worth noting that the doses of His-Leu used in our study were comparable to, or even higher than, the doses of peptides containing His-Leu in studies by others. For example, Shin and collaborators tested a tripeptide, His-His-Leu, given as a triple injection (5 mg/kg BW per injection), which reduced systolic blood pressure by 61 mmHg.⁹ In the study by Li et al., a tetrapeptide, Ala-His-Leu-Leu, was administered orally at a dose of 5 and 1 mg/kg BW daily, which resulted in a drop in systolic blood pressure

by 22.1 and 5.0 mmHg, respectively.⁸ Furthermore, doses of His-Leu tested in our study were 10–100 times higher than hypertensive doses of Ang II in rats.^{12,13} Finally, chronic haemodynamic effects of His-Leu were assessed during continuous telemetry recordings, which is the reference method for haemodynamic measurements. This method is more sensitive and reproducible than the tail-cuff technique,^{11,14} which was used in the above-mentioned studies on haemodynamic effects of Ala-His-Leu-Leu and His-His-Leu peptides.

Although in the present study His-Leu failed to affect haemodynamic parameters and plasma renin activity in rats, it cannot be excluded that this peptide may have some biological importance in the circulatory system. Arterial blood pressure is controlled by a complex network of feedback mechanisms. Therefore, it is possible that His-Leu affected some mechanisms controlling haemodynamics; however, the effect could be small enough to be masked by the counter-regulatory mechanisms.

A limitation of our study is that we tested haemodynamic effects of His-Leu administered only parenterally. It may be speculated that oral administration of the dipeptide may produce different effects. Nevertheless, to the best of our knowledge, our study is the first to investigate haemodynamic effects of His-Leu. Considering that several other dipeptides are known to have strong physiological effects, further studies on the potential effect of His-Leu in biological systems are needed.

Conclusions

Our study provides evidence for the lack of significant acute and chronic haemodynamic effects of His-Leu, a dipeptide formed in the process of converting angiotensin I into angiotensin II, in normotensive and hypertensive rats.

Acknowledgements

Conception and design of the work: LP, MU. Acquisition, analysis, or interpretation of data for the work: AD, KB, PK, MU. Drafting the work: MU, AD, KB, PK, LP. All authors have approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Medical University of Warsaw (grant numbers: 1S7/PM1/16/16 and 1S7/N/17/17).

ORCID iD

Marcin Ufnal  <https://orcid.org/0000-0003-0088-8284>

References

1. Lee SY and Hur SJ. Antihypertensive peptides from animal products, marine organisms, and plants. *Food Chem* 2017; 228: 506–517.
2. Daliri EB, Oh DH and Lee BH. Bioactive peptides. *Foods* 2017; 6: E32.
3. Ferrario CM and Mullick AE. Renin angiotensin aldosterone inhibition in the treatment of cardiovascular disease. *Pharmacol Res* 2017; 125: 57–71.
4. Drapala A, Sikora M and Ufnal M. Statins, the renin–angiotensin–aldosterone system and hypertension – a tale of another beneficial effect of statins. *J Renin Angiotensin Aldosterone Syst* 2014; 15: 250–258.
5. Perazzo J, Castanho MA and Sa Santos S. Pharmacological potential of the endogenous dipeptide kyotorphin and selected derivatives. *Front Pharmacol* 2016; 7: 530.
6. Davis CK, Laud PJ, Bahor Z, et al. Systematic review and stratified meta-analysis of the efficacy of carnosine in animal models of ischemic stroke. *J Cereb Blood Flow Metab* 2016; 36: 1686–1694.
7. Li Y, Zhou J, Huang K, et al. Purification of a novel angiotensin I-converting enzyme (ACE) inhibitory peptide with an antihypertensive effect from loach (*Misgurnus anguillicaudatus*). *J Agric Food Chem* 2012; 60: 1320–1325.
8. Li Y, Zhou J, Zeng X, et al. A novel ACE inhibitory peptide Ala-His-Leu-Leu lowering blood pressure in spontaneously hypertensive rats. *J Med Food* 2016; 19: 181–186.
9. Shin ZI, Yu R, Park SA, et al. His-His-Leu, an angiotensin I converting enzyme inhibitory peptide derived from Korean soybean paste, exerts antihypertensive activity in vivo. *J Agric Food Chem* 2001; 49: 3004–3009.
10. Bagby SP, McDonald WJ and Mass RD. Serial renin–angiotensin studies in spontaneously hypertensive and Wistar-Kyoto normotensive rats. Transition from normal- to high-renin status during the established phase of spontaneous hypertension. *Hypertension* 1979; 1: 347–354.
11. Sikora M, Konopelski P, Pham K, et al. Repeated restraint stress produces acute and chronic changes in hemodynamic parameters in rats. *Stress* 2016; 19: 621–629.
12. Huang BS, Ahmadi S, Ahmad M, et al. Central neuronal activation and pressor responses induced by circulating ANG II: Role of the brain aldosterone–“ouabain” pathway. *Am J Physiol Heart Circ Physiol* 2010; 299: H422–H430.
13. Ufnal M, Jazwiec R, Dadlez M, et al. Trimethylamine-N-oxide: A carnitine-derived metabolite that prolongs the hypertensive effect of angiotensin II in rats. *Can J Cardiol* 2014; 30: 1700–1705.
14. Wilde E, Aubdool AA, Thakore P, et al. Tail-cuff technique and its influence on central blood pressure in the mouse. *J Am Heart Assoc* 2017; 6: e005204.