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

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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

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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

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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, Vetoquinol Biowet, Poland) 100 mg/kg BW and xylazine (20 mg/ml, Xylapan, Vetoquinol Biowet, Poland) 10 mg/kg BW intraperitoneally, as previously described.11 After seven days of postsurgical 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 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 (λex = 540/λem = 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.

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 | 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 | 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 | 101.4 ± 2.8 | 344 ± 7 |
| 15 mg His-Leu/kg body weight | 104.1 ± 1.7 | 340 ± 6 |
| SHR | 163.5 ± 3.3 | 326 ± 4 |
| 15 mg His-Leu/kg body weight | 164.4 ± 1.7 | 325 ± 4 |

Table 1.
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 MAPB for the first 5 min after the infusion ($p < 0.05$). However, the between-group analysis showed no significant differences in MABP changes (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
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.

Figure 2. Changes in mean arterial blood pressure (ΔMABP (mmHg)) and heart rate (ΔHR (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.

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.\(^9\) 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.

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