Leptin, acting centrally as a neuromodulator, induces the activation of the sympathetic nervous system, which may lead to a pressor action in normotensive animals. In haemorrhagic shock, leptin administered intracerebroventricularly (icv.) evokes the resuscitating effect, with long-lasting rises in mean arterial pressure (MAP) and heart rate (HR), subsequent increase in peripheral blood flows, and a 100% survival at 2 h. Since leptin is able to activate histaminergic neurons, and centrally acting histamine also induces the resuscitating effect with the activation of the sympathetic nervous system, in the present study, we investigated an involvement of the histaminergic system in leptin-evoked cardiovascular effects in haemorrhagic shock. The model of irreversible haemorrhagic shock, with MAP decreased to and stabilised at 20 – 25 mmHg, has been used. Leptin (20 µg) given icv. at 5 min of critical hypotension evoked 181.5% increase in extracellular hypothalamic histamine concentration during the first 10 min after injection. Rises in MAP, HR and renal, mesenteric and hindquarters blood flows induced by leptin were inhibited by icv. pre-treatment with histamine H₁ receptor antagonist chlorpheniramine (50 nmol). In contrast, there was no effect of H₂, H₃, and H₁ receptor antagonists ranitidine (25 nmol), VUF 5681 (25 nmol) and JNJ 10191584 (25 nmol), respectively. In conclusion, the histaminergic system is involved in centrally-acting leptin-induced resuscitating effect in haemorrhagic shock in rats.

**Key words:** histaminergic system, leptin, haemorrhagic shock, mean arterial pressure, heart rate, histamine receptor antagonist

---

**INTRODUCTION**

Haemorrhagic shock is a life-threatening condition resulting from blood loss, and the cardiovascular response to haemorrhage can be divided into three phases. The first one is characterized by an increase of the sympathetic nervous system activity, with tachycardia and a rise in the peripheral resistance. In the second phase, the activation of unmyelinated vagal afferents from the left ventricle initiates a decrease in the sympathetic activity, with a fall in the peripheral resistance and bradycardia. Finally, continuous blood loss leads to a further decrease in blood pressure accompanied by tachycardia (1).

Experimental studies demonstrate an influence of many neuronal systems, including opioidergic (2), melanocortinergic (3), serotonergic (4), cholinergic (5) and histaminergic system (6), on the sympathoinhibitory phase of regulation in critical hypovolaemia. Histaminergic neurons are concentrated at the tuberomammillary nuclei of the hypothalamus and send axons to many parts of the central nervous system influencing circadian rhythms, thermoregulation, learning and memory, nociceptive responses, feeding behaviour, hypothalamic and pituitary hormone secretion and cardiorespiratory control (7). In normotension, histamine acting as a neurotransmitter induces an increase in mean arterial pressure (MAP) with bradycardia in conscious and tachycardia in anaesthetised animals (8). Interestingly, in critical haemorrhagic hypotension, histamine evokes a long-lasting and a few fold higher pressor effect in comparison to normotensive rats, with an improvement in survival (6). We demonstrated an involvement of the sympathetic nervous system, the renin-angiotensin system, as well as of arginine vasopressin (AVP) and proopiomelanocortin (POMC)-derived peptides in histamine-induced resuscitating action (9). Haemodynamic mechanisms responsible include the mobilisation of blood from venous reservoirs leading to a partial normalisation of peripheral blood flows and blood gas and acid-base parameters (10-11).

Leptin is a peptide hormone secreted by adipocytes which decreases energy consumption by reducing appetite and thermogenesis (12). The former effect is associated with centrally-mediated activation of the sympathetic nervous system, which is not restricted to the adipose tissue, and concerns also the cardiovascular system (13). Experimental studies demonstrate that after administration into the third cerebral ventricle or lateral ventricle (icv.), the peptide evokes a long-lasting increase in MAP in normotensive conscious (13) and anaesthetised rats (14). Moreover, in a rat model of irreversible haemorrhagic shock,
leptin induces a long-lasting pressor effect with the increase in survival, and the mechanism includes the activation of the sympathetic nervous system (15). Since leptin, acting as a neuromodulator, is able to enhance the activity of histaminergic neurons (16), and both histamine and leptin have common mechanism involved in the resuscitating action, the purpose of the study was to examine the involvement of the histaminergic system in leptin-mediated cardiovascular effects in haemorrhagic shock.

MATERIAL AND METHODS

Animals

All procedures were performed in accordance to the EU directives and reviewed by the local Ethics Committees (Katowice, Poland and Bursa, Turkey; notifications No 23/2010, 09/2011, 28/2012, 29/2012, 84/2014 and 2009-11/03). Studies were performed in male Wistar rats weighing 230 – 284 g (5 – 6 months old). The animals were housed in individual cages in the animal colony, under standard, controlled conditions (temperature 20 – 22°C, humidity 60 – 70%, 12 h light/dark cycle) and provided with food and water ad libitum.

Surgical preparation

After induction of general anaesthesia with ketamine/xylazine (100 mg/kg + 10 mg/kg intraperitoneally, supplemented if required), rats were implanted with catheters filled with heparinised saline (300 IU/ml) in the right carotid artery and the right jugular vein. MAP and heart rate (HR) were measured using TAM-A transducer amplifier module and ECGA amplifier (Hugo Sachs Elektronik, Germany), respectively. The electromagnetic perivascular probes (type 1RB and 2.5SB, Hugo Sachs Elektronik, Germany) were implanted around the right renal and superior mesenteric arteries to monitor renal (RBF) and mesenteric (MBF) blood flow and around the distal abdominal aorta, below the ileocecal artery, to monitor perfusion of the hindquarters (HBF) using TTFM transit time flowmeter module (Transonic Systems Inc., USA). All measurements of blood flow were started after a 30 min adaptation period to avoid influences of probe implantation. Regional vascular resistances were calculated by dividing MAP (mmHg) by regional blood flows (ml/min).

For i.c.v. treatment, rats were prepared 5 – 7 days before the experiment by stereotaxic implantation, under ketamine/xylazine anaesthesia, of polyethylene cannulae into the right brain lateral ventricle as previously described (6). All icv. injections were made in the volume of 5.0 µl. Correctness of injections was verified as previously described (6).

Experimental protocol

Severe haemorrhagic shock, according to the method by Guarini et al. (3), was produced by intermittent blood withdrawal from the catheter inserted into the right jugular vein over a period of 15 – 25 min, until MAP decreased to and stabilised at 20 – 25 mmHg.

Immediately after induction of critical MAP, the animals were pre-treated icv. with the histamine H1/H2 receptor antagonists chlorpheniramine (50 nmol), ranitidine (25 nmol), VUF 5681 (25 nmol) and JNJ 10191584 (25 nmol), respectively, or saline. Five min later, rats were injected icv. with leptin (20 µg) or saline. Doses of leptin and histamine H1/H2 receptor ligands were taken from the literature (6, 15, 17). VUF 5681 and JNJ 10191584 were administered at an equimolar dose to previously used thiopearamide, H1 receptor inverse agonist/H4 antagonist (18). Animals were continuously monitored for 2 hours after treatment, or until death, if it occurred earlier. Body temperature was monitored by a rectal thermometer and maintained at 37 ± 0.5°C using heating lamps throughout experiment. All the experiments were performed between 8.00 and 14.00.

According to recommendations of the Local Ethics Committee in Katowice, to avoid the duplication of experiments performed at our laboratory with the same rat strain, using the same experimental protocol and with the same H1 and H2 receptor antagonists (6, 17), we did not repeat experiments in control saline icv.-treated groups and cited and discussed previously published results, especially that the mortality at 2 h in these groups was 100%.

Microdialysis study

Handmade microdialysis probes (by Burcin Altinbas) were used. Anaesthetised and catheterised rats were placed in a stereotaxic frame. The skull was exposed and drilled over the posterior hypothalamus (coordinates: 3.6 mm posterior to bregma, 0.5 mm lateral (right) to the midline and 9.0 mm vertical to the skull). Probes (molecular weight cut-off dialysis membrane was 18,000 Da and length was 2.0 mm) were implanted and then fixed with acrylic cement to the skull.

At the end of the probe placement, the arterial catheter was connected to a transducer for MAP monitoring, and the microdialysis probe was attached to perfusion pump. The dialysis probe was perfused with artificial cerebrospinal fluid (pH 7.4) of the following composition: 120 mmol/l NaCl, 1.3 mmol/l CaCl2, 1.2 mol/l MgSO4, 1.2 mmol/l NaHPO4, 3.5 mmol/l KCl, 25 mmol/l NaHCO3, and 10 mmol/l glucose. The perfusion rate was 2 µl/min. The dialysis probe was perfused for the first 60 min of the stabilisation period and samples were collected at 10 min intervals. After this period, one more sample was collected before haemorrhage and this sample was measured as basal histamine level. Collection of microdialysis samples was continued 70 min after the start of bleeding. At the end of the experiments, the animals were decapitated, and for the determination of microdialysis probe location, brains were fixed in 4% paraformaldehyde, and 50 micrometer-thick vibratom sections were collected. Sections were stained with haematoxylin-eosin for 15 min. Excess stain was washed off in distilled water and the sections were dehydrated by rinses through graduated ethanol series. Following the cleaning step in xylene, the sections were coverslipped using dispersive pipet extraction. Probe localization was determined and representative pictures (Fig. 1) were taken using an Olympus BX-50 microscope adapted with a CCD digital camera.

High-performance liquid chromatography (HPLC) measurement of histamine levels

Extracellular histamine levels at the posterior hypothalamus were measured by HPLC (Jasco PU-980 Intelligent HPLC pump; Kipp & Zonen printer) and UV detection (Jasco UV-975 Intelligent UV-VIS detector) at a wavelength of 220 nm using a C18 column (Hypersil C18; 5 µm; 25 cm; 4.6 mm ID) with an isocratic system (0.04 mol/l KH2PO4; 0.015 mol/l sodium-1-hepatosulfonate and 25% acetonitrile, pH 3.1). Flow rate was 2.0 ml/min. Chromatograms were completed within 10 min.

Drugs

The following drugs were used: leptin (rat, recombinant) (Sigma-Aldrich, USA), chlorpheniramine maleate, ranitidine hydrochloride (Research Biochemicals Incorporated, USA), JNJ 10191584, VUF 5681 (Tocris Bioscience, UK), ketamine
hydrochloride, xylazine (Biowet Sp. z o. o., Poland), heparin (Polfa, Poland). All drug solutions were prepared freshly on the day of the experiment.

**Statistical analysis**

All values are given as means ± standard deviation, with P < 0.05 considered as the level of significance. The Fisher’s exact test was used to examine statistical differences in survival percentage. Histamine levels were compared by Kruskal-Wallis test, whereas statistical evaluation of the other results was performed using analysis of variance (ANOVA) and the post-ANOVA test of Student-Newman-Keuls.

**RESULTS**

The initial pre-bleeding values of MAP, pulse pressure (PP), HR and peripheral blood flows in all groups did not reveal significant differences.

The total bleeding volume necessary for the induction of critical MAP in all animals was 2.43 ± 0.35 ml/100 g body weight. In the control saline ivc. injected group, bleeding from MAP 85.3 ± 6.56 mmHg to 23.11 ± 1.12 mmHg was associated with a decrease in PP from 43.76 ± 5.85 mmHg to 15.32 ± 3.7 mmHg and HR from 324 ± 18 beats/min to 210 ± 23 beats/min (Fig. 2). Furthermore, haemorrhage led to a decrease in RBF from 4.97 ± 0.78 ml/min to 0.87 ± 0.22 ml/min, HBF from 10.84 ± 1.48 ml/min to 2.35 ± 0.65 ml/min and MBF from 6.75 ± 1.29 ml/min to 1.29 ± 0.12 ml/min (Fig. 3). Initial renal (RVR), hindquarters (HVR) and mesenteric vascular resistance (MVR) in the control saline-injected group were: 17.35 ± 2.0 mmHg/ml/min, 7.95 ± 1.33 mmHg/ml/min and 12.2 ± 1.64 mmHg/ml/min, respectively (Fig. 4). After shock induction, vascular resistances were increased to 27.5 ± 3.6 mmHg min/ml/ml, 11.0 ± 3.0 mmHg/ml/min and 22.8 ± 4.9 mmHg/ml/min, respectively (Fig. 4).

**Influence of histamine receptor ligands on haemodynamic effects of leptin in haemorrhage-shocked rats**

Leptin administered to haemorrhage-shocked rats induced an increase in MAP, PP and HR (Fig. 2), which were accompanied by a rise in peripheral blood flows (Fig. 3). The effects started within 10 – 15 min after leptin injection, were long-lasting and associated with 100% survival rate at 2 h (P < 0.05 versus control saline-treated animals, Fisher’s exact test). Since the bleeding termination to the end of the observation period peripheral vascular resistances were increased (Fig. 4). In the saline-pre-treated group, 20 min after leptin injection RVR, HVR and MVR were still increased by 56.4%, 35.9% and 115.3%, respectively, in comparison with the pre-bleeding values (Fig. 4).

In the saline-treated control group, there were no increases in MAP, PP, HR (Fig. 2) and peripheral blood flows (Fig. 3), and all animals died within 30 min.

*Fig. 1. Photomicrograph of a typical placement of microdialysis probe in the posterior hypothalamus; the arrow indicates the tip of the microdialysis probe; 3V - third ventricle; haematoxylin-eosin staining.*
Chlorpheniramine inhibited MAP, PP and regional haemodynamic effects of leptin (Figs. 2-4), however, without influence on HR and survival rate at 2 h. The antagonist given alone, as we showed previously (6), had no influence on haemodynamic parameters in the icv. saline-treated control group.

On the other hand, H_2, H_3 and H_4 receptor blockers ranitidine, VUF 5681 and JNJ 10191584 did not influence measured cardiovascular parameters (Figs. 2-3) and calculated peripheral vascular resistances (Fig. 4) in leptin-treated group. The antagonists given alone in the control groups, as we reported previously with ranitidine (17) and studied here with VUF 5681 and JNJ 10191584 (data not shown), had no influence on measured haemodynamic parameters and calculated peripheral vascular resistances.
Influence of leptin on histamine release in the posterior hypothalamus in critically hypotensive rats

In vivo microdialysis studies showed that the basal extracellular histamine concentrations at the posterior hypothalamus in control and leptin-treated groups were 0.14 ± 0.03 pmol/10 min and 0.133 ± 0.08 pmol/10 min, respectively (Fig. 5). Induction of critical haemorrhagic hypotension did not significantly influence histamine concentrations (Fig. 5).

Intracerebroventricular administration of leptin (20 µg) evoked 181.5% increase in extracellular histamine levels at the posterior hypothalamus during the first 10 min after injection (Fig. 5). On the contrary, in the control group there were no significant changes in the posterior hypothalamic histamine levels within 10 min after saline treatment (Fig. 5).

DISCUSSION

Centrally acting leptin influences cardiovascular regulation both in normotension (13, 19) and critical haemorrhagic hypotension (15). Present results extend previous studies and show for the first time the influence of leptin on the activity of histaminergic neurones in critical hypovolaemia. However, the main novel finding in this study is the direct demonstration of the involvement of the histaminergic system in leptin-mediated resuscitating effect in haemorrhage-shocked rats.

Due to a low permeability of the blood-brain barrier, similarly to previous studies (15), we administered leptin centrally, although the influence of circulating leptin on the central cardiovascular regulation in haemorrhagic shock cannot be excluded, since i) in focal and global cerebral hypoxia/traumatic brain injury the blood-brain permeability increases (20), ii) the effect is potentiated during oxidative stress (21) which characterises the reperfusion phase of haemorrhagic shock resuscitation and iii) peripherally administered leptin induces neuroprotective effects on focal cerebral ischemia/reperfusion injury (22).

As we confirmed here, leptin given at a dose 20 µg icv. to rats subjected to critical haemorrhagic hypotension induces a long-lasting and significantly higher increase in MAP, relative to normotensive animals (15) with a reversal of bradycardia and improvement of the survival rate at 2 h, which is considered as a long-lasting survival. The action of leptin is accompanied by the rise in RBF, MBF and HBF, as demonstrated since 10 min after

**Fig. 4.** RVR (A), HVR (B) and MVR (C) before and after bleeding termination, and 20 min after icv. injection with leptin (L, 20 µg) in groups pre-treated with chlorpheniramine (CHL, 50 nmol), ranitidine (RAN, 25 nmol), VUF 5681 (VUF, 25 nmol), JNJ 10191584 (JNJ, 25 nmol), and in the control saline-injected (S) group. Means ± S.D., 6 animals per group. *P < 0.05 versus pre-bleeding value; **P < 0.05 versus corresponding value in saline-pre-treated leptin injected group.

**Fig. 5.** Hypothalamic extracellular histamine concentrations before bleeding, 10 and 20 min after the start of bleeding and 10 – 40 min after icv. leptin (20 µg) or saline injections. Means ± S.D., 4 animals per group. *P < 0.05 versus saline-treated group. There are no results of histamine levels in the control group at 20, 30 and 40 min of critical hypovolaemia, because all animals died before the collections.
stimulating hormone (αMSH) (25). On the other hand, αMSH release in vivo mediates the pressor effect. diphenhydramine blocks leptin-evoked increases in MAP and iliac diphosphocholine (17) and the agonist of 5-HT3 receptor 8-OH-DPAT (33). Moreover, pre-treatment with chlorpheniramine decreases leptin-induced increase in MAP and iliac vasoconstriction (15), and the H3 receptor antagonist diphenhydramine blocks leptin-evoked increases in MAP and the renal sympathetic nerve activity in normotensive rats (33).

To further determine the role of the histaminergic system in the central leptin-induced resuscitating action, we used histamine receptor ligands. Haemodynamic results show that H3 receptors are involved in leptin-mediated resuscitating effect in haemorrhagic shock. These data are in line with the previous studies in which we demonstrated the role of H4 receptor in the resuscitating effect of centrally acting endogenous and exogenous histamine (9). H5 receptors are involved also in the mediation of the pressor effect of centrally acting cytidine 5′-diphosphocholine (17) and the agonist of 5-HT3 receptor 8-OH-DPAT (33). Moreover, pre-treatment with chlorpheniramine decreases leptin-induced increase in MAP and iliac vasoconstriction (15), and the H3 receptor antagonist diphenhydramine blocks leptin-evoked increases in MAP and the renal sympathetic nerve activity in normotensive rats (33).

According to the published literature, we suggest indirect mechanisms since leptin is able to influence the activity of other neuronal pathways involved in leptin-mediated cardiovascular effects, although both in conscious (13) and anaesthetized normotensive rats (14) centrally acting leptin induces a pressor effect. Although we demonstrated the involvement of the histaminergic system in the central cardiovascular regulation in shock (18), here we did not find any influence of central H2, H3 and H4 receptor blockade on leptin-mediated effects. Presynaptic H1 receptors mediate autoinhibition of histamine release from the histaminergic neurons as well as regulation of the synthesis/release of other neurotransmitters (7). Tanida et al. (34) demonstrated that H3 receptor antagonist/inverse agonist tiotropium inhibits changes in the renal sympathetic nerve activity and MAP evoked by low doses of histamine in normotensive rats. In contrast, VUF 5681, a silent/neutral H3 receptor antagonist did not affect measured cardiovascular parameters in shock. We hypothesize that the difference can be explained by the pharmacological properties of the used H3 ligands.

In conclusion, the results of our studies directly demonstrate the involvement of the histaminergic system in the central cardiovascular regulation in shock, there are limitations of our study. In addition to the melancortinergic and histaminergic systems, centrally acting leptin is able to influence the activity of other neuronal pathways, and their role in the observed resuscitating effect cannot be excluded. The study by Garcia et al. (36) showed a leptin-mediated increase in the central thyrotropin-releasing hormone (TRH) synthesis and release, and TRH evokes an increase in the sympathetic tone in normotensive animals (37) and induces a resuscitating effect in experimental haemorrhagic shock (38). Moreover, leptin is involved in AVP synthesis and secretion (32), and AVP belongs to essential compensatory pathways in haemorrhagic shock (39). Finally, we did not study particular neuronal pathways activated by leptin. We injected it i.v., similarly as it was performed in the studies concerning the function of other poorly penetrating the blood-brain barrier neurotransmitters/neuromodulators (40-42).

In conclusion, the results of our studies directly demonstrate for the first time the involvement of the histaminergic system in centrally-acting leptin-induced resuscitating effect in haemorrhagic shock in rats.

Acknowledgements: The study was supported by a grant of the Medical University of Silesia, Katowice, Poland (KNW-1-065/P/20) and by COST Action BM0806 “Recent advances in histamine receptor H4R research”. We thank Dr M. Ozgur Ozyigit for the technical assistance at histological procedures.

Conflict of interests: None declared.
REFERENCES


30. Ye ZY, Li DP. Activation of the melanocortin-4 receptor causes enhanced excitation in presymptomatic paraventricular neurons in obese Zucker rats. Regul Pept 2011; 166: 112-120.


Received: May 3, 2015
Accepted: January 23, 2016

Author’s address: Prof. Jerzy Jochem, Medical University of Silesia, Katowice, Department of Basic Medical Sciences, 18 Piekarska Street, 41-902 Bytom, Poland.
E-mail: jjochem@poczta.onet.pl