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INVOLVEMENT OF THE HISTAMINERGIC SYSTEM IN THE RESUSCITATING EFFECT OF CENTRALLY ACTING LEPTIN IN HAEMORRHAGIC SHOCK IN RATS

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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 H_1 receptor antagonist chlorpheniramine (50 nmol). In contrast, there was no effect of H_2, H_3 and H_4 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 increasing energy expenditure, possibly *via* increased 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 ileocaecal 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 H_1-H_4 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 H_1-H_2 receptor ligands were taken from the literature (6, 15, 17). VUF 5681 and JNJ 10191584 were administered at an equimolar dose to previously used thioperamide, H_3 receptor inverse agonist/H_4 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 H_1 and H_2 receptor antagonists (6, 17), we did not repeat experiments in the 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 $CaCl_2$, 1.2 mol/l $MgSO_4$, 1.2 mmol/l NaH_2PO_4, 3.5 mmol/l KCl, 25 mmol/l $NaHCO_3$ 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 (HLPC) 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 KH_2PO_4, 0.015 mol/l sodium-1-heptasulfonate 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

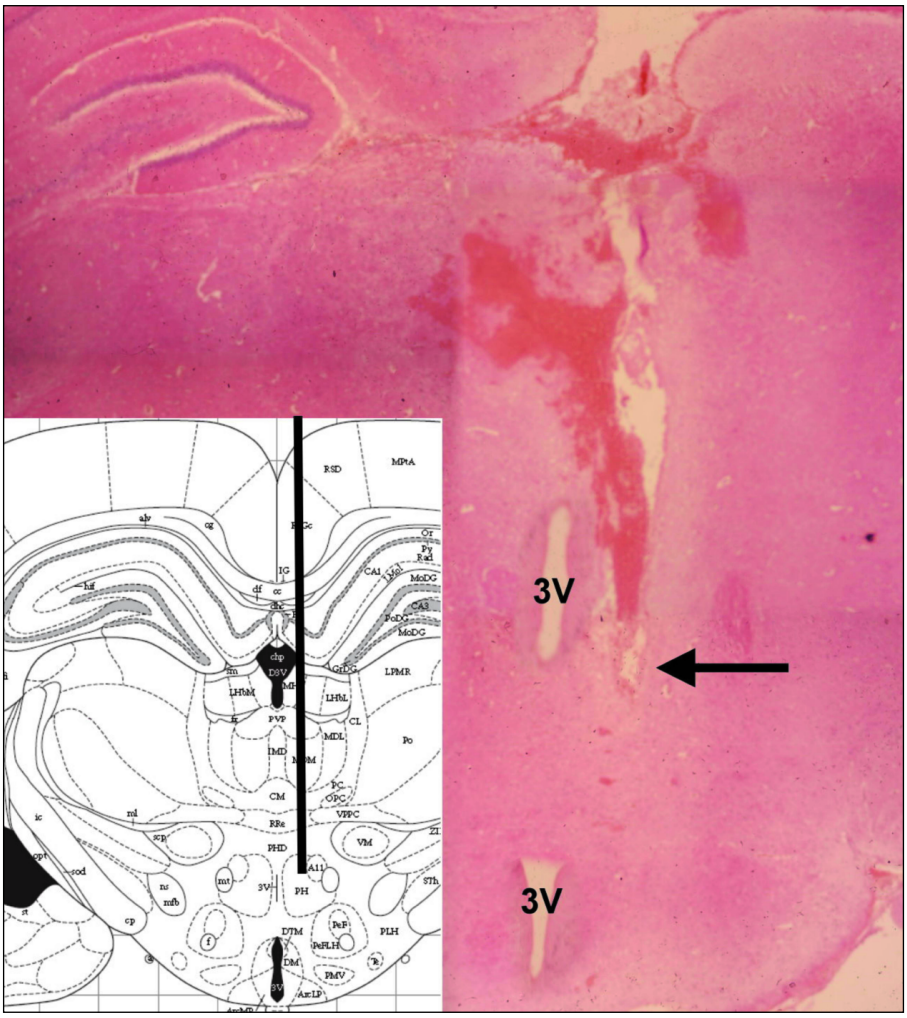

Fig. 1. Photomicrograph of a typical placement of microdialysis probe in the posterior hypothalamus; the arrow indicates the tip of the microdialysis probe; 3V - third ventricle; haematoxylin-eosin staining.

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 icv. 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 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 we no increases in MAP, PP, HR (*Fig. 2*) and peripheral blood flows (*Fig. 3*), and all animals died within 30 min.

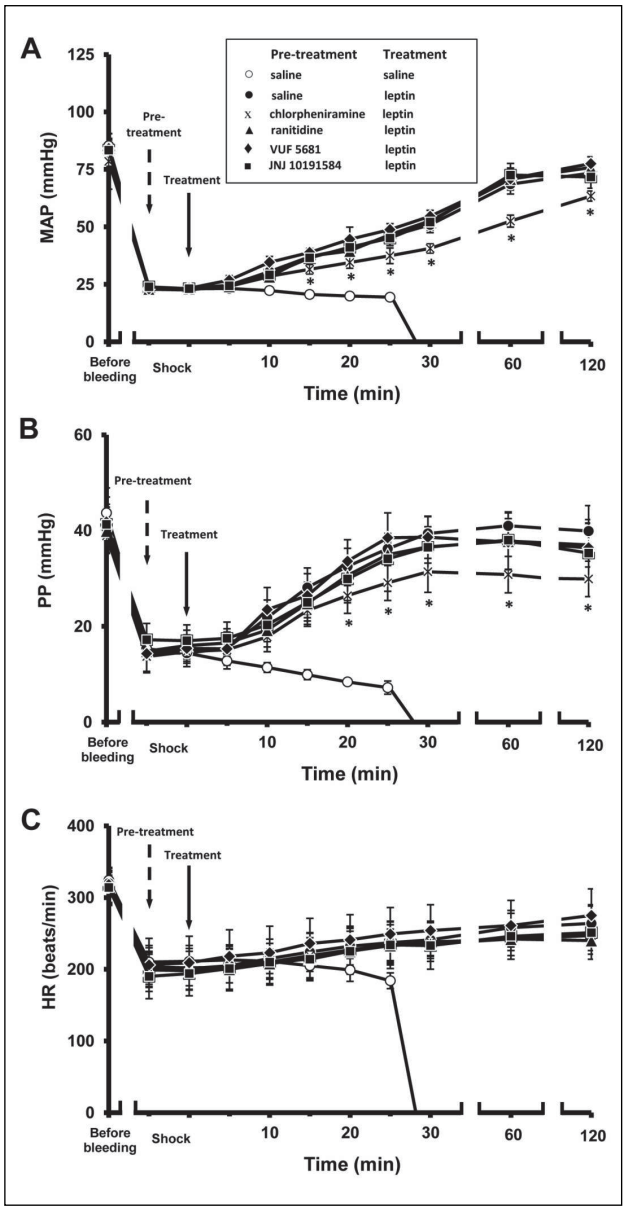

Fig. 2. Influence of icv. pre-treatment with chlorpheniramine (50 nmol), ranitidine (25 nmol), VUF 5681 (25 nmol), JNJ 10191584 (25 nmol) and saline (5 µl) on MAP (A), PP (B) and HR (C) after leptin (20 µg, icv.) administration in rats subjected to critical haemorrhagic hypotension. Means ± S.D., 6 animals per group. In all leptin-treated groups, since 10 min (MAP, PP) and 25 min (HR) till the death of control animals $P < 0.05$ versus the saline-treated control group; *$P < 0.05$ versus the saline-pre-treated leptin-injected group; previous (6, 17) and present studies with histamine receptor ligands (data not shown) did not evoke MAP, PP and HR changes after their administration in the control groups.

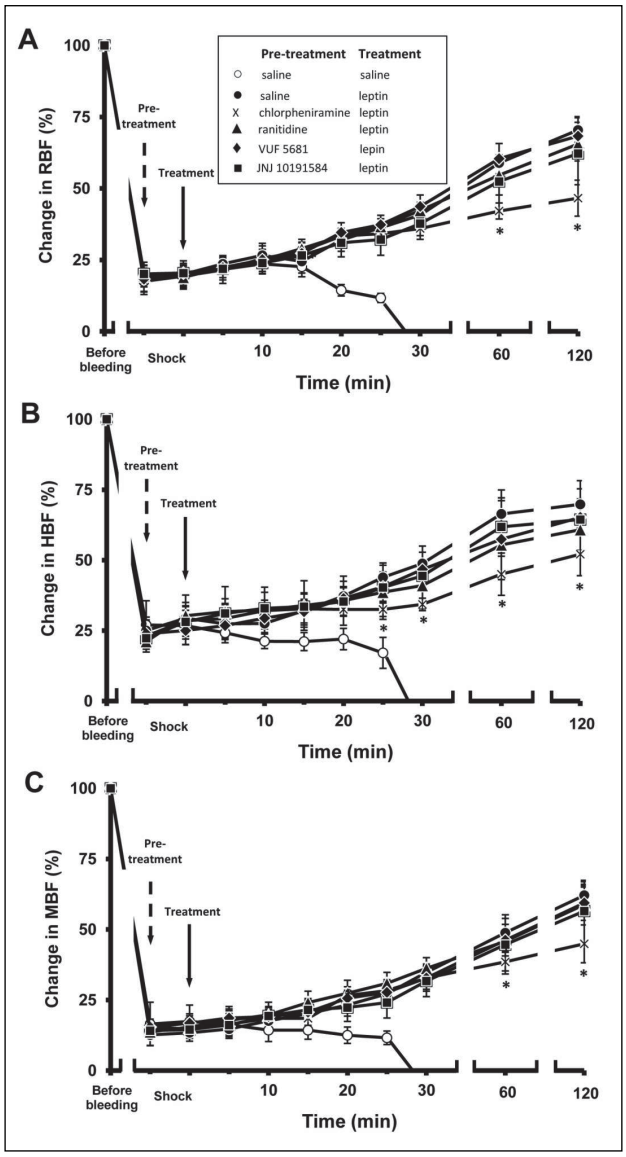

Fig. 3. Influence of icv. pre-treatment with chlorpheniramine (50 nmol), ranitidine (25 nmol), VUF 5681 (25 nmol), JNJ 10191584 (25 nmol) and saline (5 µl) on RBF (A), HBF (B) and MBF changes (C) after leptin (20 µg, icv.) administration in rats subjected to critical haemorrhagic hypotension. Means ± S.D., 6 animals per group. Pre-bleeding values of RBF, HBF and MBF in the control group were: 4.97 ± 0.78 ml/min, 10.84 ± 1.48 ml/min and 6.75 ± 1.29 ml/min, respectively. In all leptin-treated groups, since 10 min (HBF), 15 min (MBF) and 20 min (RBF) till the death of control animals $P < 0.05$ versus the saline-treated control group; *$P < 0.05$ versus the saline-pre-treated leptin-injected group; previous (6, 17) and present studies with histamine receptor ligands (data not shown) did not reveal peripheral blood flow changes after their administration in the control groups.

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.

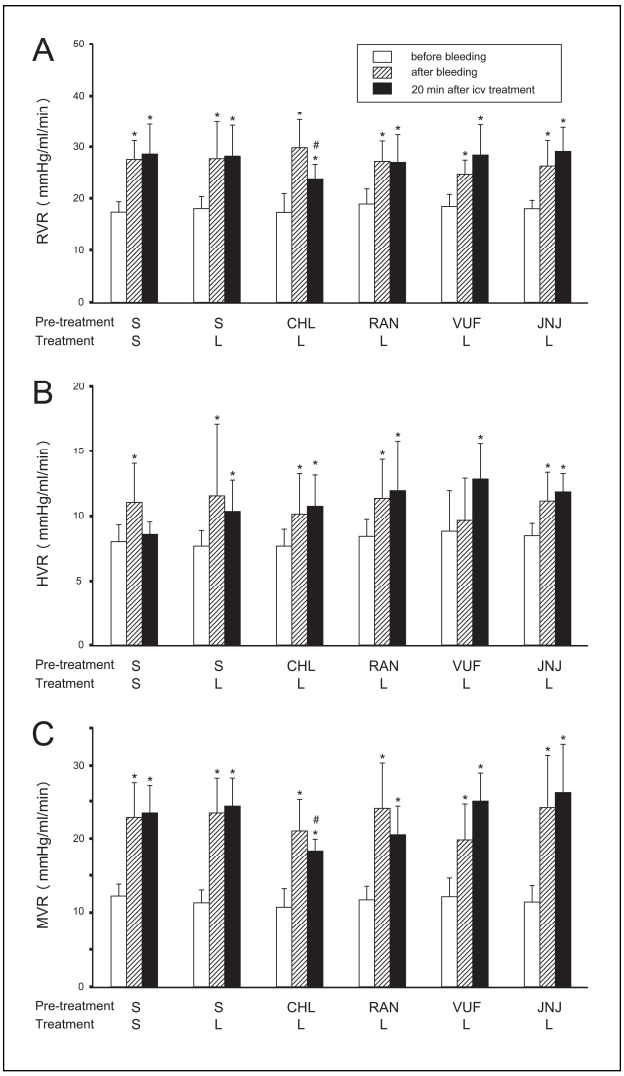

Fig. 4. RVR (A), HVR (B) and MVR (C) before and after bleeding termination, and 20 min after icv. injection with leptin (L, 20 μg) in groups pre-treated with chlorpheniramine (CHL, 50 nmol), ranitidine (RAN, 25 nmol), VUF 5681 (VUF, 25 nmol), JNJ 10191584 (JNJ, 25 nmol), and in the control saline-injected (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.

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

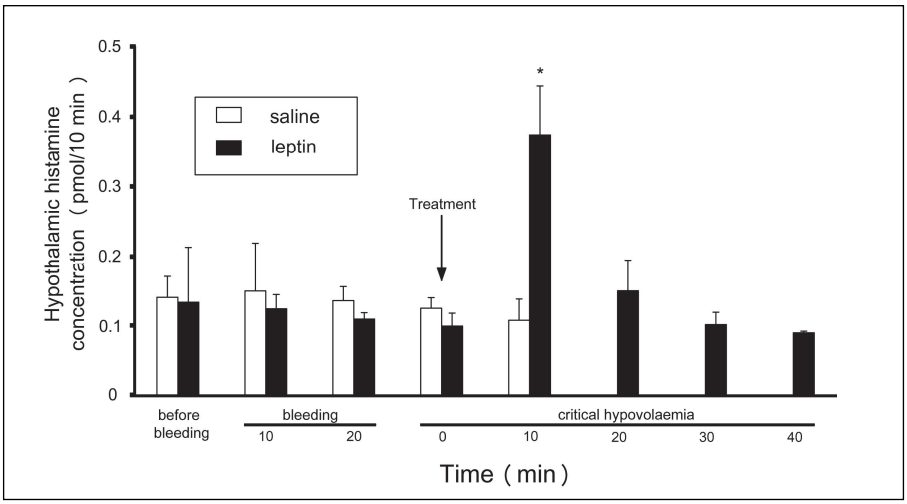

Fig. 5. Hypothalamic extracellular histamine concentrations before bleeding, 10 and 20 min after the start of bleeding and 10 – 40 min after icv. leptin (20 μg) or saline injections. Means ± S.D., 4 animals per group. *P < 0.05 versus saline-treated group. There are no results of histamine levels in the control group at 20, 30 and 40 min of critical hypovolaemia, because all animals died before the collections.

treatment (15). We hypothesized that the haemodynamic mechanism responsible for shock reversal is related to the increase in the circulating blood volume, as a consequence of blood mobilisation from venous reservoirs, that in turn leads to the increase in peripheral blood flows. This hypothesis is supported by the finding of a long-lasting vasoconstriction, especially in the mesenteric region (15). The differences among RVR, MVR and HVR can be explained by the specificity of the sympathetic innervations of these vascular beds, as well as the different vascular density of receptors for endogenous vasoconstrictors. Similar differences in regional peripheral blood flows and vascular resistances were described earlier in studies regarding cardiovascular effects of the histaminergic system activation (9) and the treatment with AVP (23) in haemorrhagic shock.

Our data may suggest a potential direction of a new adjunctive pharmacological intervention in haemorrhagic shock, however, it is difficult to assess their clinical significance, especially that i) leptin was given centrally, ii) we used a model of isolated haemorrhagic shock, whereas in humans it is often accompanied by polytrauma and iii) anaesthesia, volume replacement and other standard therapeutics used to treat haemorrhagic shock might interfere with leptin action in hypovolaemia. Experimental studies demonstrate the influence of anaesthesia on cardiovascular regulation in hypovolaemia i.e. the shortening of the sympathoexcitatory phase (24). In the present studies, all injections of leptin were performed in the second, sympathoinhibitory phase, however, we cannot exclude a possible influence of anaesthesia on leptin-mediated cardiovascular effects, although both in conscious (13) and anaesthetized normotensive rats (14) centrally acting leptin induces a pressor effect.

According to the model used, the animals were monitored for 2 hours and in that time leptin evoked an improvement in measured cardiovascular parameters and survival rate. However, since the main mechanism of action is the activation of the sympathetic nervous system (15) associated with an increase in energy expenditure, further studies are needed to fully assess a long-lasting metabolic effects of leptin action.

Central neuronal pathways involved in leptin-mediated resuscitating action in haemorrhagic shock are not clear. According to the published literature, we suggest indirect mechanisms since leptin is able to stimulate neurons located in the arcuate nucleus and projecting to the paraventricular nucleus (PVN) of the hypothalamus, where they release α-melanocyte stimulating hormone (α-MSH) (25). On the other hand, *in vivo* electrophysiological studies demonstrate that leptin activates the sympathetic nervous system by stimulating α-MSH release in PVN (19). Since α-MSH and other POMC-derived peptides have protective effects due to the activation of the cholinergic anti-inflammatory pathway in haemorrhagic shock, multiple organ dysfunction syndrome and other critical conditions (26-28), we hypothesize that the leptin-induced resuscitating effect may be mediated *via* POMC-derived peptides; further, the central melanocortinergic system favourably influences the sympathetic tone and, this way, MAP values, as results of *in vivo* (29) and *in vitro* (30) studies demonstrate. In addition, findings by Fekete and Liposits (31) show that the α-MSH-containing neurons of the arcuate nucleus directly innervate histaminergic neurons in the tuberomammillary nucleus and therefore, may be involved in the mediation of leptin action on this neuronal system. Indeed, *in vivo* microdialysis studies demonstrate an increase in histamine release at the anterior hypothalamus after intraperitoneal administration of leptin in normotensive anaesthetised rats (32) and mice (16). In the present study, we demonstrate for the first time leptin-induced increase in hypothalamic histamine release in haemorrhage-shocked animals.

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 H_1 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 H_1 receptor in the resuscitating effect of centrally acting endogenous and exogenous histamine (9). H_1 receptors are involved also in the mediation of the pressor effect of centrally acting cytidine 5'-diphosphocholine (17) and the agonist of 5-HT_{1A} receptor 8-OH-DPAT (33). Moreover, pre-treatment with chlorpheniramine decreases leptin-induced increase in MAP and iliac vasoconstriction (15), and the H_1 receptor antagonist diphenhydramine blocks leptin-evoked increases in MAP and the renal sympathetic nerve activity in normotensive rats (33).

Similarly to our previous studies concerning the role of the histaminergic system in the central cardiovascular regulation in shock (18), here we did not find any influence of central H_2, H_3 and H_4 receptor blockade on leptin-mediated effects. Presynaptic H_3 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 $H_{3/4}$ receptor antagonist/inverse agonist thioperamide 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 H_3 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 H_3 ligands.

Despite a single report on the functional expression of histamine H_4 receptors in the mammalian brain (35), we showed no influence of the H_4 receptor antagonist JNJ 10191584 on the central cardiovascular regulation in leptin-injected and saline-treated animals. These results are in agreement with our previous studies in which thioperamide did not affect the action of cholinergic (17) and serotonergic ligands (18) in haemorrhagic shock.

Although we demonstrated the involvement of the histaminergic system in leptin-mediated cardiovascular effects in shock, there are limitations of our study. In addition to the melanocortinergic 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 icv., 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.

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Conflict of interests: None declared.

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