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INVOLVEMENT OF THE RENIN-ANGIOTENSIN SYSTEM IN ENDOGENOUS CENTRAL HISTAMINE-INDUCED REVERSAL OF CRITICAL HAEMORRHAGIC HYPOTENSION IN RATS

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The study was undertaken to examine the involvement of the renin-angiotensin system in the reversal by endogenous central histamine of critical haemorrhagic hypotension in anaesthetised Wistar rats. Histamine N-methyltransferase inhibitor metoprine (20 µg) administered intracerebroventricularly at 5 min of critical hypotension 20–25 mmHg produced increases in histamine concentrations as measured 20 min after treatment in the hypothalamus (581.33 ± 63.23 vs. 488.26 ± 56.34 ng/g of wet tissue; $P < 0.01$) and medulla oblongata (53.42 ± 14.65 vs. 34.68 ± 13.52 ng/g of wet tissue; $P < 0.05$). That was accompanied by 34.7% higher plasma angiotensin II concentration in comparison to the control group. Metoprine produced dose-dependent (5–20 µg) rises in mean arterial pressure (MAP) and heart rate, which were significantly higher than those in normotensive animals. The resuscitating action of metoprine (20 µg) was associated with rises in renal, mesenteric and hindquarters blood flows, and a 100% survival at 2 h after treatment, while in the saline-treated group, all the animals died within 30 min. Angiotensin type 1 (AT₁) receptor antagonist ZD 7155 (0.5 mg/kg; *iv*) decreased regional vascular resistance and inhibited metoprine-induced increase in MAP, whereas AT₂ receptor blocker PD 123319 (10 mg/kg; *iv*) had no effect. Angiotensin-converting enzyme inhibitor captopril (30 mg/kg; *iv*) reduced the increase in plasma angiotensin II level and the haemodynamic effects of metoprine. Neither captopril, nor angiotensin receptor antagonists influence the survival at 2 h after treatment. In conclusion, the renin-angiotensin system is involved in central histamine-induced resuscitating action in rats.

Key words: *haemorrhagic shock, histamine, metoprine, renin-angiotensin system, rats*

INTRODUCTION

Histamine, acting as a neurotransmitter, influences a number of the central nervous system functions, such as arousal mechanisms, fluid balance, food intake, temperature regulation, learning, pain perception and cardiovascular regulation (1-2). There is an increase in endogenous histamine release from the posterior hypothalamus during haemorrhage in cats (3), which suggests activation of the histaminergic system in hypotension. Moreover, previous studies by the author show that both endogenous and exogenous histamine acting centrally produces the resuscitating action in haemorrhage-shocked rats (4-6). These results confirm the hypotheses that the activation of the histaminergic system is associated with response to the action of adverse or potentially dangerous stimuli disturbing homeostasis (2) and, in these conditions, histamine may participate in mobilisation of compensatory mechanisms (4-6).

In normotensive rats, inhibition of histamine N-methyltransferase (HNMT; EC 2.1.1.8) activity, leading to an increase in endogenous central histamine concentrations due to the blockage of its catabolism, produces a dose-dependent pressor effect (4, 7). Similarly, exogenous histamine administered into the brain lateral ventricle (*icv*), acting *via* the central noradrenergic system (8), evokes short-lasting rises in mean arterial pressure (MAP) and heart rate (HR) (6, 9). Both central H₁ and H₂ histamine receptors are involved independently in cardiovascular effects, however, the pressor action is mediated mainly *via* H₁, and HR changes *via* H₂ receptors (10).

Previous studies by the author reveal that exogenous histamine (10-100 nmol; *icv*) as well as endogenous histamine, after treatment with HNMT inhibitor SKF 91488 (10-100 µg; *icv*), acting *via* central H₁ receptors, is able to reverse the critical haemorrhagic hypotension and to increase the survival rate at 2 h in a rat model of irreversible pressure-controlled haemorrhagic shock (4, 6). Interestingly, MAP and HR changes elicited by histamine in the early phase of haemorrhagic hypotension are long-lasting and significantly higher compared to those in normovolaemic animals (4, 6). The differences in response to central histamine between normovolaemic and critically-shocked rats suggest that, in addition to the central activation of the sympathetic system and the release of arginine vasopressin (AVP) which mediate histamine-induced cardiovascular effects in normotension (1, 2), there are other mechanisms involved in the state of critical hypotension. Since the central histamine is able to increase the secretion of proopiomelanocortin (POMC)-derived peptides, such as adrenocorticotrophic hormone (ACTH), β-endorphin, β-lipotropin and α-melanocyte stimulating hormone (α-MSH), and to activate the renin-angiotensin system (2), it is postulated that these mechanisms can also be associated with the resuscitating effect.

The present paper, being a continuation of previous studies on the role of the histaminergic system in the central cardiovascular regulation in haemorrhagic

shock (4-6), demonstrates the involvement of the renin-angiotensin system in the reversal of critical hypotension. Since the tissue renin-angiotensin system plays little part in the protection against hypotension (11), plasma angiotensin II concentrations were measured as an indicator of the circulating renin-angiotensin system activation. The experimental haemorrhagic shock model by Guarini *et al.* (12) was chosen, as in the previous studies (4-6, 13), to examine histamine action at constant initial values of both critical MAP and the volume of circulating blood.

MATERIALS AND METHODS

Animals

Studies were carried out in 78 male Wistar rats weighing 250-320 g (5-6 months old). The animals were housed in individual cages in the animal colony, under controlled conditions of temperature (20-22°C), humidity (60-70%), lighting (12 h light/dark cycle) and provided with food and water *ad libitum*. Each studied group consisted of six animals. All experimental procedures were performed according to the EU directives and reviewed by the Ethics Committee of the Medical University of Silesia (Notification No 5/03).

Surgical preparations

For *icv* treatment the rats were prepared 5-7 days before the experiment by implantation, under ketamine/xylazine (100 mg/kg/10 mg/kg; *ip*) anaesthesia, of polyethylene cannula into the right brain lateral ventricle (6). All *icv* injections were made in a volume of 10 μ l over a period of 60 s, and the correctness of injections was verified as described previously (6).

On the day of the experiment, after induction of general anaesthesia with ethylurethane (1.25 g/kg; *ip*), the rats were implanted with catheters filled with heparinized saline (300 IU/ml) in the right carotid artery and the right jugular vein.

Cardiovascular parameter measurements

MAP and HR were measured using the pressure transducer RMN-201 (Temed, Zabrze, Poland) and the electrocardiograph Diascope 2 (Unitra Biazet, Białystok, Poland), respectively. Electromagnetic probes (Type 1RB2006, Hugo Sachs Elektronik, March-Hugstetten, Germany) were implanted around the right renal and the superior mesenteric arteries to monitor renal (RBF) and mesenteric (MBF) blood flow, and around the distal abdominal aorta, below the level of the ileocaecal artery, to monitor perfusion of the hindquarters (HBF) using Transit Time Flowmeter Type 700 (Hugo Sachs Elektronik, March-Hugstetten, Germany) (14). Regional vascular resistance was calculated by dividing MAP (mmHg) by regional blood flow (ml/min). All measurements of blood flow were started after a 30 min adaptation period to avoid influences of probe implantation.

Haemorrhagic shock protocol

Irreversible haemorrhagic shock, according to the method of Guarini *et al.* (12), was produced by intermittent blood withdrawal from the catheter inserted into the right jugular vein over a period of 15-25 min, until MAP stabilised at 20-25 mmHg.

Experimental protocol

To study cardiovascular effects of HNMT blockage, metoprine (5, 10, 20 µg; *icv*) was injected in three groups of haemorrhage-shocked rats 5 min after termination of bleeding, and in three groups of normovolaemic animals. Since a dose of 20 µg of metoprine produced a long-lasting pressor effect and a 100% survival at 2 h, it was used for the further studies.

To examine the role of the renin-angiotensin system in histamine-induced reversal of critical hypotension, in separate groups, metoprine (20 µg; *icv*) was administered 5 min after termination of bleeding after pre-treatment with:

- angiotensin-converting enzyme (ACE) inhibitor captopril (30 mg/kg; *iv* 30 min earlier),
- selective non-peptide angiotensin type 1 (AT₁) receptor antagonist ZD 7155 (0.5 mg/kg; *iv* 5 min earlier),
- selective non-peptide angiotensin type 2 (AT₂) receptor blocker PD 123319 (10 mg/kg; *iv* 5 min earlier),
- saline (0.2 ml).

Since preliminary experiments demonstrated no cardiovascular differences between animals treated *icv* with metoprine-vehicle and 0.9% saline solution, in the control groups, the rats were injected centrally with saline solution. All antagonist doses were taken from the literature (15-17).

The animals were continuously monitored for 2 h after treatment, or until death, if it occurred earlier. Body temperature was monitored by a rectal thermometer and maintained at $37 \pm 0.5^\circ\text{C}$ using a heating lamp throughout the experiment. All the experiments were performed between 8 a.m. and 2 p.m.

Histamine assay

Endogenous central histamine concentrations were measured in two groups of haemorrhage-shocked rats 20 min after treatment with metoprine (20 µg; *icv*) or saline. After decapitation of the rats, their brains were rapidly removed and the cerebral cortex, hypothalamus and medulla oblongata were quickly dissected on a glass plate chilled on ice, according to the procedure of Glowinski and Iversen (18). The samples were homogenised by sonication - the hypothalamus in 0.3 ml, the cerebral cortex and medulla oblongata in 10 volumes (w/v) of ice-cold 0.9% NaCl. After centrifugation (3000 rpm for 20 min at 4°C), 100 µl of the supernatant was used for measurement of histamine concentration by commercially available enzyme immunoassay (Immunotech, Marseille, France). The sensitivity of the method was 0.2 nmol/l. The mean recovery of standard histamine was 93% (ranging from 87 to 107%) (4).

Determination of plasma angiotensin II concentration

To measure plasma angiotensin II concentrations, 0.8 ml of blood was withdrawn from the arterial catheter before bleeding, and before and 20 min after *icv* treatment with metoprine (20 µg) or saline, as well as from animals pre-treated with captopril and metoprine-injected, to ice-cold polypropylene tubes containing EDTA. The volume was replaced with 0.9% solution of NaCl. Blood samples were centrifuged immediately (1800 rpm for 10 min at 4°C), plasmas were separated and frozen at -80°C. Plasma angiotensin II levels were measured using commercially available EIA kit (Phoenix Pharmaceuticals, Inc., Belmont, CA, USA). The sensitivity of the method was 0.06 ng/ml.

Drugs

The following drugs were used: metoprine (Burroughs Wellcome Co., Research Triangle Park, NC, USA), ZD 7155 hydrochloride [5,7-diethyl-3,4-dihydro-1-[[2'-(1*H*-tetrazol-5-yl)]1,1'-

biphenyl]-4-yl)methyl]-1,6-naphthyridin-2(1*H*)-one hydrochloride], PD 123319 ditrifluoroacetate [1-[[4-(dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic acid ditrifluoroacetate] (Tocris Cookson Inc., Ellisville, MO, USA), xylazine hydrochloride (Research Biochemicals Inc., Natick, MA, USA), captopril (Sigma Chemical Co., St. Louis, MO, USA), ethylurethane (Riedel-de Haën, Seelze, Germany), ketamine (Gedeon Richter, Budapest, Hungary), lactic acid, NaHCO₃ (POCH, Gliwice, Poland) and heparin (Polfa, Warszawa, Poland). To avoid stimulation of the osmoreceptors all solutions were isoosmolar to the plasma. Osmolarity was measured using osmometer Marcel OS 3000 (Marcel, Białystok, Poland) based on the freezing-point method. All the injected agents, except metoprine, were dissolved in hypotonic solutions of NaCl; metoprine was dissolved in 0.5% lactic acid and was neutralised with NaHCO₃. All drug solutions were prepared freshly on the day of the experiment.

Statistics

All values are given as means \pm standard deviation with $P < 0.05$ considered as the level of significance. Statistical evaluation was performed by analysis of variance (ANOVA) and the post-ANOVA test of Neuman-Keules. The Fisher's exact test was used to examine significant differences in survival rates.

RESULTS

The baseline pre-bleeding values of MAP and HR in all groups did not reveal significant differences. Similarly, there were no differences among the groups with respect to peripheral blood flows and vascular resistance.

The total bleeding volume for the induction of critical hypotension in all animals was 2.30 ± 0.22 ml/100 g body weight. In the control saline *icv* pre-treated group, bleeding from MAP 83.6 ± 6.4 mmHg to 20-25 mmHg was associated with a decrease in HR from 345 ± 8 beats/min to 223 ± 14 beats/min.

Concentrations of histamine in the cerebral cortex, hypothalamus and medulla oblongata

There were significantly higher histamine concentrations in metoprine (20 μ g)-treated animals than in the saline-treated group as measured 20 min after *icv* injection in the hypothalamus (581.33 ± 63.23 vs. 488.26 ± 56.34 ng/g of wet tissue; $P < 0.01$) and medulla oblongata (53.42 ± 14.65 vs. 34.68 ± 13.52 ng/g of wet tissue; $P < 0.05$) (*Fig. 1*). In contrast, cerebral cortical histamine levels did not differ between the two groups (51.04 ± 17.23 vs. 44.91 ± 19.14 ng/g of wet tissue).

Plasma angiotensin II concentrations

There were no differences between the saline- and metoprine-treated groups in initial plasma angiotensin II concentrations (*Fig. 2*). Five min after termination of bleeding, angiotensin II levels in both groups were 4.1- and 3.9-fold higher in comparison to the pre-bleeding values ($P < 0.05$ vs. pre-bleeding values), respectively. At 20 min after *icv* injections, there were further increases in plasma

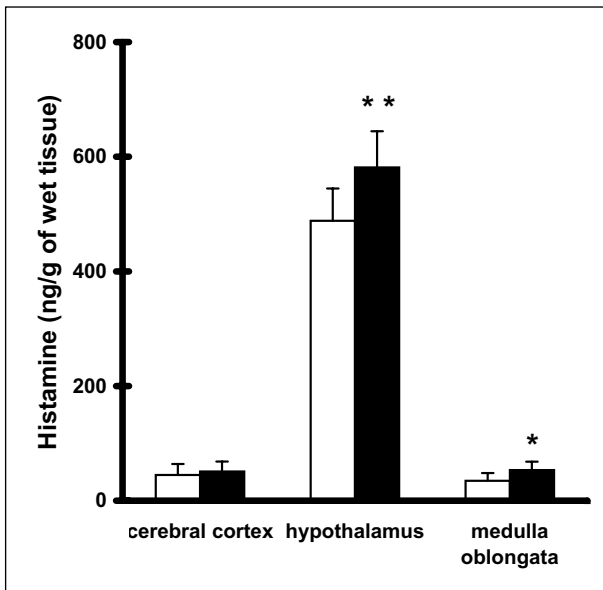


Fig. 1. Concentrations of histamine in the cerebral cortex, hypothalamus and medulla oblongata in haemorrhage-shocked rats 20 min after *icv* treatment with saline (open columns) and metoprine (20 µg, filled columns). Data are represented as mean ± SD; six animals per group; * P < 0.05, ** P < 0.01 vs. saline-treated group

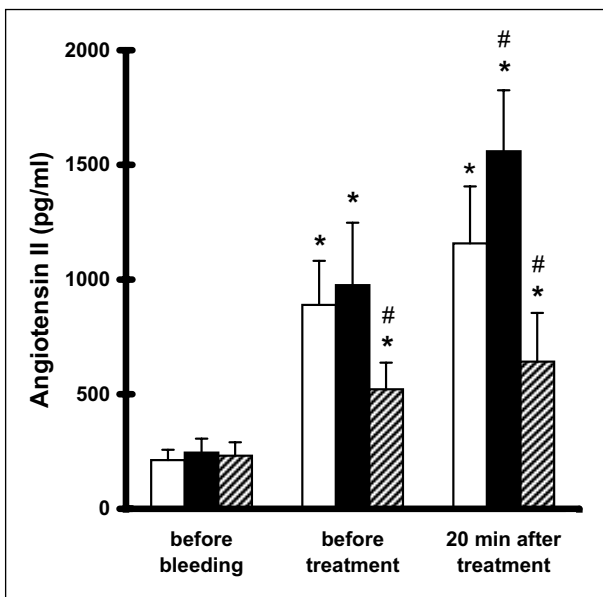


Fig. 2. Plasma angiotensin II concentrations before bleeding, before and 20 min after *icv* treatment with saline (open columns), metoprine (20 µg, filled columns), and metoprine (20 µg) after pre-treatment with captopril (30 mg/kg, cross-hatched square). Data are represented as mean ± SD; six animals per group; * P < 0.05 vs. baseline value, # P < 0.05 vs. saline-treated group

angiotensin II concentrations (Fig. 2), however, in the metoprine-injected group the level was higher by 34.7% in comparison to the control group (P < 0.05).

Pre-treatment with captopril (30 mg/kg; *iv*) significantly inhibited rises in angiotensin II concentrations before and 20 min after metoprine treatment (Fig. 2).

Cardiovascular effects of metoprine in normotensive and critically hypotensive rats

In normotensive animals, metoprine (5-20 μg ; *icv*) produced dose-dependent increases in MAP (Fig. 3A) and HR (Fig. 3B) which started within 5-20 min after injection. The pressor effect was long-lasting, and HR, but not MAP, returned to the initial values within 2 h after injection.

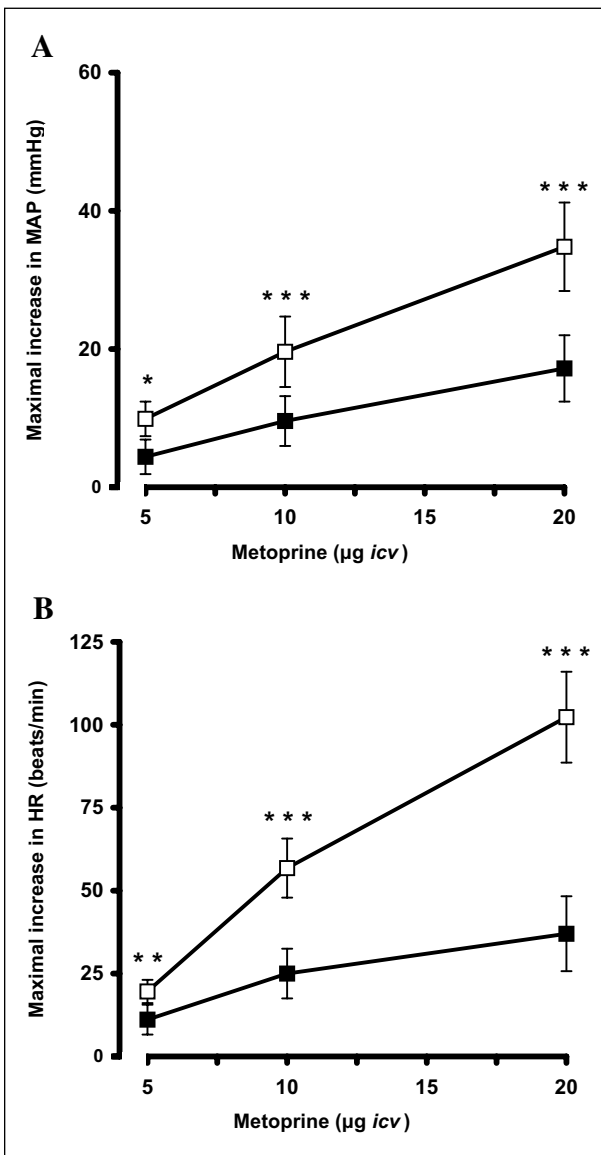


Fig. 3. Metoprine-induced maximal changes in mean arterial pressure (MAP; A) and heart rate (HR; B) within 2 h after *icv* treatment in normotensive (■) and critically hypotensive (□) rats. Data are represented as mean \pm SD; six animals per dose. Baseline MAP and HR values in all normotensive animals were 85.3 ± 4.9 mmHg and 359 ± 12 beats/min, respectively. Baseline MAP and HR values in critically hypovolaemic rats were 22.3 ± 1.2 mmHg and 238 ± 21 beats/min, respectively. * P < 0.05, ** P < 0.01, *** P < 0.001 vs. normotensive rats

Metoprine administered to rats bled to a critical hypotension caused dose-dependent increases in MAP (*Fig. 3A*) and HR (*Fig. 3B*) which were 2.0- to 2.2- and 1.7- to 2.8-fold higher compared to those in normovolaemic rats, respectively. Metoprine at a dose of 5 μg produced only transient increases in MAP and HR, and the survival rate of 2 h was 33.33%. In contrast, metoprine given at doses of 10 and 20 μg evoked long-lasting rises in MAP and HR, and the increase in the survival rates at 2 h to 83.33% and 100% ($P < 0.05$ vs. the control saline-treated animals; Fisher's exact test), respectively.

Influence of captopril and angiotensin receptor antagonists on regional haemodynamic effects of metoprine and the survival rate

Changes in MAP, HR, regional haemodynamics and vascular resistance over time in the metoprine (20 μg)- and saline-treated groups are presented in *Fig. 4-6*. Haemorrhage produced in the control saline-injected group decreases in RBF

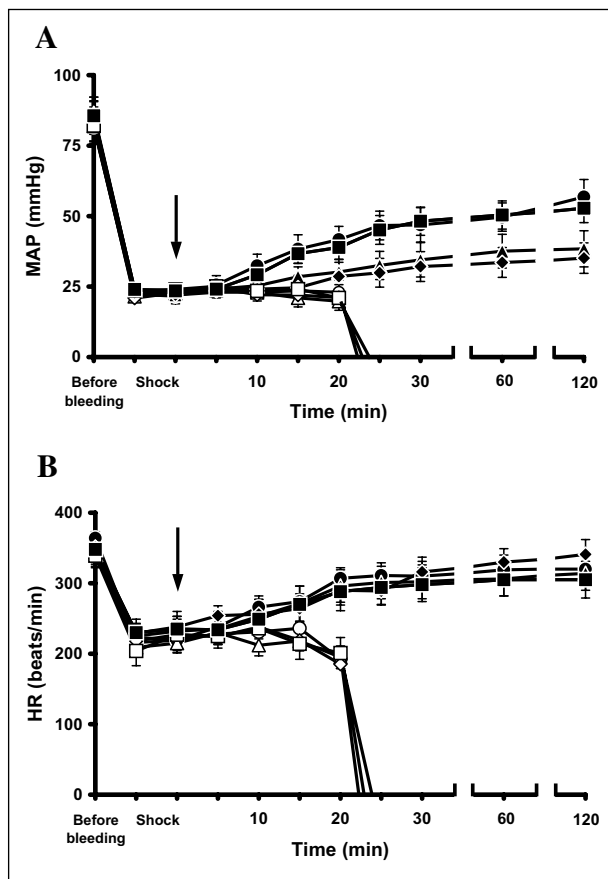


Fig. 4. Influence of *iv* pre-treatment (-5 min) with saline (●, ○), captopril (30 mg/kg; ▲, △), ZD 7155 (0.5 mg/kg; ◆, ◇) and PD 123319 (10 mg/kg; ■, □) on mean arterial pressure (MAP; A) and heart rate (HR; B) after treatment (0 min; arrow) with metoprine (20 μg ; filled symbols) and saline (5 μl ; open symbols). Data are represented as mean \pm SD, six animals per group. For MAP, since 15 min in all groups, except the PD 123319-pre-treated metoprine-injected animals, $P < 0.05$ vs. the saline-pre-treated metoprine injected group. For HR, since 15 min in all saline-treated groups, $P < 0.05$ vs. the saline-pre-treated metoprine injected group

from 6.61 ± 0.98 ml/min to 0.87 ± 0.33 ml/min, HBF from 8.56 ± 1.55 ml/min to 0.89 ± 0.41 ml/min and MBF from 7.21 ± 1.91 ml/min to 0.66 ± 0.23 ml/min, with no differences between the groups.

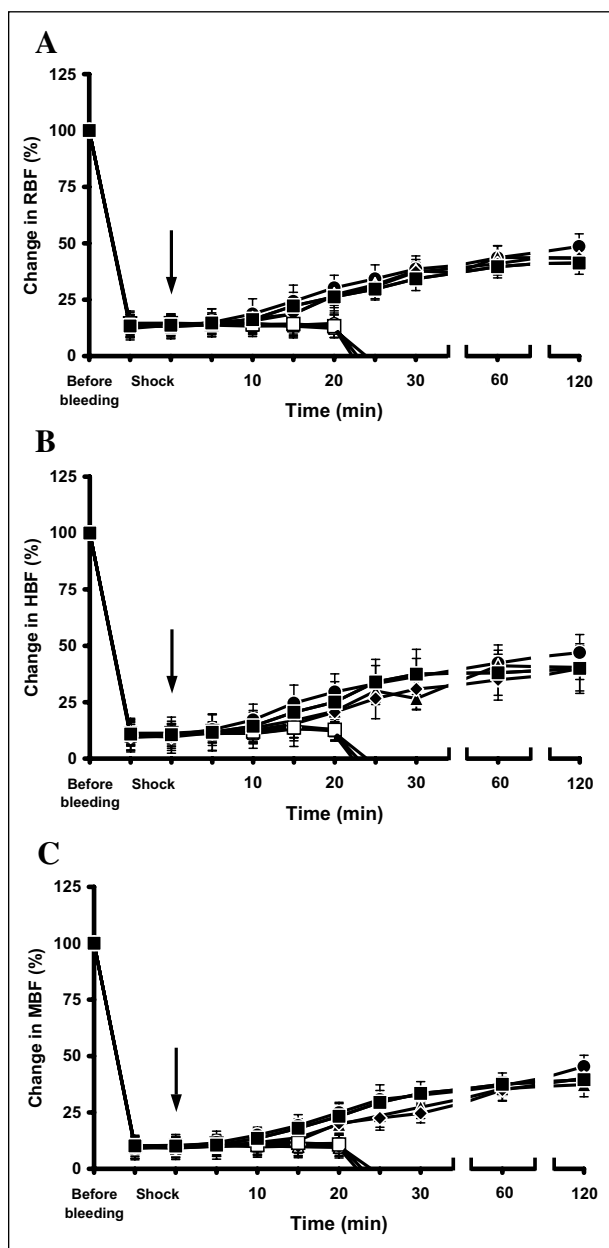


Fig. 5. Influence of *iv* pre-treatment (-5 min) with saline (\bullet , \circ), captopril (30 mg/kg; \blacktriangle , \triangle), ZD 7155 (0.5 mg/kg; \blacklozenge , \lozenge) and PD 123319 (10 mg/kg; \blacksquare , \square) on regional blood flows changes after treatment (0 min; arrow) with metoprine (20 μ g; filled symbols) and saline (5 μ l; open symbols). Data are represented as percent of the initial pre-bleeding value, mean \pm SD are given; six animals per group. Initial renal (RBF; A), hindquarters (HBF; B) and mesenteric blood flow (MBF; C) in the saline-treated control group are 6.61 ± 0.98 ml/min, 8.56 ± 1.55 ml/min and 7.21 ± 1.91 ml/min, respectively. Since 15 min for all saline-treated groups $P < 0.05$ vs. saline-pre-treated metoprine-injected group

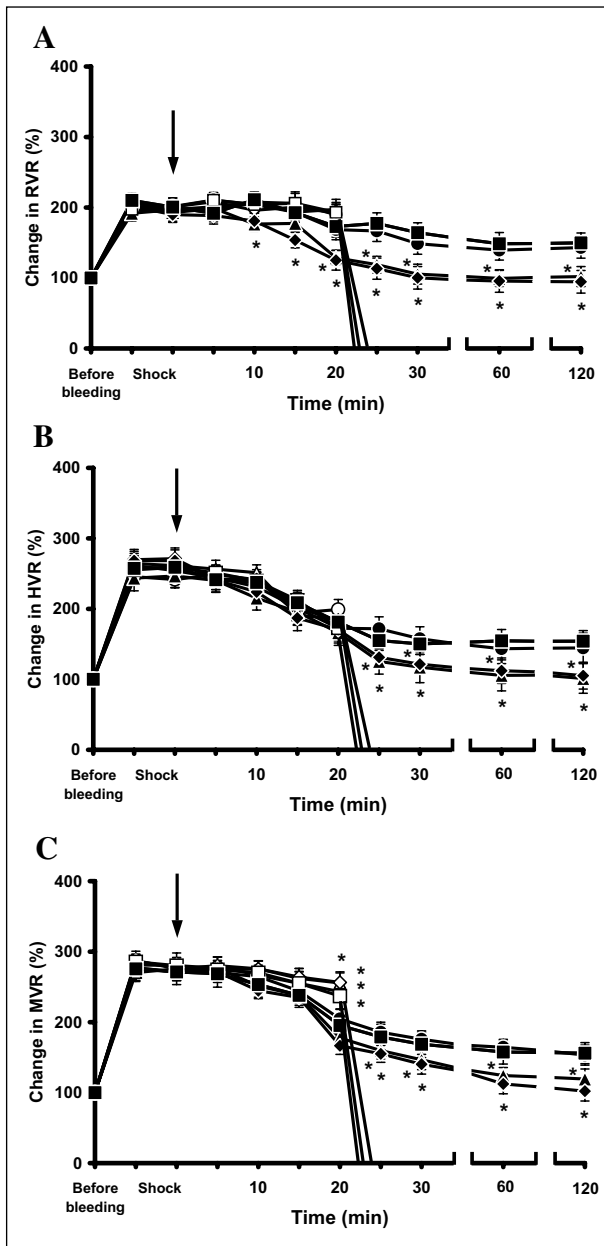


Fig. 6. Influence of *iv* pre-treatment with saline (●, ○), captopril (30 mg/kg; ▲, △), ZD 7155 (0.5 mg/kg; ◆, ◇) and PD 123319 (10 mg/kg; ■, □) on regional vascular resistance after treatment (0 min; arrow) with metoprine (20 μg; filled symbols) and saline (5 μl; open symbols). Data are represented as percent of the initial pre-bleeding value, mean ± SD are given; six animals per group. Initial renal (RVR; A), hindquarters (HVR; B) and mesenteric vascular resistance (MVR; C) in the saline-treated control group are 12.64 ± 3.52 mmHg/ml/min, 9.76 ± 2.43 mmHg/ml/min and 11.59 ± 3.95 mmHg/ml/min, respectively. * P < 0.05 vs. saline-pre-treated metoprine-injected group

In the saline-pre-treated group, metoprine produced long-lasting rises in MAP (Fig. 4A) and HR (Fig. 4B), and the action was associated with increased RBF, MBF and HBF (Fig. 5A-C) and decreased regional vascular resistance (Fig. 6A-

C), which started within 10 min after treatment and persisted until the end of the experiment (2 h).

Pre-treatment with ZD 7155 decreased regional vascular resistance, which led to a decrease in MAP, however, with no influence on HR (*Fig. 4-6*) and the survival rate (100%) in comparison to the metoprine-treated group. In contrast, PD 123319 did not affect metoprine-induced cardiovascular action (*Fig. 4-6*) and the survival rate (100%). Both antagonists given alone had no effect in haemorrhage-shocked control animals, and the survival (0%) was not different in comparison to the saline-treated group.

In the metoprine-injected group, captopril decreased MAP, which resulted from a lower regional vascular resistance. Inhibition of ACE activity did not influence HR and the survival rate (100%) in comparison to the saline-pre-treated metoprine-injected group. Pre-treatment with captopril in the saline *icv*-treated group had no influence on cardiovascular parameters and survival compared to the control saline *iv*-injected animals (*Fig. 4-6*).

DISCUSSION

The present results confirm that metoprine produces an increase in central histamine concentrations, which is associated with the resuscitating effect in anaesthetised rats. Moreover, it is demonstrated for the first time the involvement of the renin-angiotensin system in endogenous central histamine-induced haemodynamic effects in critical haemorrhagic hypotension.

Inhibition of HNMT activity belongs to generally accepted pharmacological methods used to explore *in vivo* the biological effects of the histaminergic system (1-2). Metoprine has previously been used to study the role of endogenous central histamine in pain perception (19), regulation of body water balance (20), anxiety (21), and learning and memory processes (22). It inhibits HNMT activity in the rat brain by more than 80% after intraperitoneal (*ip*) administration (10-20 mg/kg) (23), and produces about a 90% increase in brain histamine concentration 1 h after an oral dose (10 mg/kg) (24). The present study confirms that metoprine increases histamine levels in the hypothalamus and medulla oblongata 20 min after *icv* injection. Interestingly, there are no differences between the groups in respect to cortical histamine levels, which can be explained by insufficient penetration of metoprine to the cerebral cortex within a 20 min period after injection. In comparison to the results by Lecklin *et al.* (20), who have measured central histamine concentrations 2 h after *ip* administration of metoprine (20 mg/kg), cortical and hypothalamic histamine levels after metoprine administration in the present study are lower. It is suggested that the reasons are differences in experimental protocols, i.e. doses, different time periods of metoprine action and routes of administration (20).

Metoprine induces both in normotensive and critically hypotensive rats, dose-dependent pressor effects with increases in HR. Similarly, in conscious rats, metoprine (20 mg/kg; *ip*) produces over a 27% increase in systolic blood pressure, as measured 5 h after treatment, however, with no influence on HR (20). Present results are also consistent with the previous findings demonstrating that SKF 91488 produces increases in MAP and HR, which are significantly higher in hypovolaemic than in normovolaemic animals (4). The possible explanation is that activation of the histaminergic system leads to mobilisation of compensatory mechanisms in response to critical hypotension. The previous study based on a rat model of blood volume/blood pressure-controlled haemorrhagic hypotension demonstrates that SKF 91488, at a dose which does not influence cardiovascular parameters in normovolaemic rats, produces an increase in blood volumes necessary to induce hypotension of 40 and 20 mmHg (25). On the other hand, pre-treatment with histidine decarboxylase (EC 4.1.1.22) inhibitor (S)- α -fluoromethylhistidine, which induces a decrease in central histamine levels, reduces the volumes of blood required to achieve critical MAP 20-25 mmHg (5).

Although metoprine penetrates the blood-brain barrier after peripheral administration well, in the present experiments it has been administered centrally for two reasons. Firstly, to reduce the time necessary to penetrate the central nervous system and to shorten the latency period to evoke the action. Secondly, to avoid a possible influence of metoprine-induced increases in peripheral histamine levels. It is shown that histamine influences vascular resistance and the heart function not only directly, *via* H₁ and H₂ receptors, but also, indirectly, *via* H₃ receptors, regulates the function of the sympathetic system (26, 27). Since renal sympathetic nerves tone belongs to essential mechanisms of renin secretion (28), histamine may indirectly influence the renin-angiotensin system activity. In addition, histamine belonging to neuroendocrine mechanisms, acting directly *via* H₂ receptors, stimulates renin release (29).

Among different models of haemorrhagic shock, which can be based either on uncontrolled bleeding (30), predetermined bleeding volumes (31), fixation of predetermined blood pressure over a variable time range (32) or predetermined oxygen deficit (33), the pressure-controlled model used by Guarini *et al.* (12) is a model of severe irreversible hypotension. Previous studies demonstrate that it is associated with an early initiation of the sympathoinhibitory phase with bradycardia, development of hypoxemia and severe metabolic acidosis (34-36). The sympathoinhibitory phase of regulation in haemorrhagic shock is characterised by activation of humoral mechanisms, such as AVP, adrenal catecholamines and the renin-angiotensin system (37). Indeed, there is demonstrated over a 4.1-fold higher plasma angiotensin II concentration in the post-bleeding period in the control group. Interestingly, an increase in endogenous central histamine concentrations leads to a further increase in the plasma angiotensin II level, which confirms the hypothesis that central histamine may participate in the activation of the renin-angiotensin system. Therefore, the

present results are in agreement with the results of Kjaer *et al.*, who have demonstrated that activation of the central H₁ and H₂ receptors in rats leads to increase in plasma renin activity (PRA) (38). Moreover, pre-treatment with (S)- α -fluoromethylhistidine inhibits the dehydration-induced rise in PRA by 80% (38). The influence of histaminergic neurones on PRA changes is mediated *via* the sympathetic system since it is inhibited by peripheral β -adrenoceptor blockage (39).

Critical hypotension of 20-25 mmHg is associated with an extreme decrease in cardiac output and an increase in total peripheral resistance index (14). The effect is accompanied by extremely decreased RBF, HBF and MBF in the post-bleeding period due to increased peripheral vascular resistance as indicated in the used model of irreversible haemorrhagic shock (14). The present study shows rises in regional blood flows after metoprine treatment, which may be associated with increased volume of circulating blood (14). Indeed, centrally administered histamine in haemorrhagic shock evokes over a 100% increase of circulating blood volume 20 min after treatment, and both splenectomy and ligation of the suprahepatic veins diminish the effect (14). That suggests that there is a mobilisation of blood from venous reservoirs in response to central histamine. On the other hand, an increase in peripheral tissue perfusion in a late phase of shock may result from decreased responsiveness to endogenous vasoconstrictive factors, which is associated with endothelins-mediated release of NO and/or cyclooxygenase products which influence the vascular reactivity (40-41).

To study the role of the renin-angiotensin system in central histamine-induced haemodynamic effects in critical hypotension, ACE inhibitor and selective angiotensin receptor antagonists have been administered 5 min before metoprine treatment. Both captopril and ZD 7155 inhibit metoprine-induced increases in MAP, however, without influence on the survival at 2 h. The effect is due to a decrease in renal (RVR), hindquarters (HVR) and mesenteric vascular resistance (MVR). It is previously demonstrated that in normotensive rats angiotensin II constricts primarily the renal vasculature and, to a lesser extent, the gut circulation (15). The present study shows a decrease not only in MVR, but also in RVR and HVR, after AT₁ receptor blockage. On the other hand, PD 123319 has no effect on haemodynamics in shock, which is consistent with the study by Champion *et al.*, who demonstrate that only AT₁ receptors are involved in cardiovascular regulation in rats (17).

The present study demonstrates that captopril not only prevents an increase in plasma angiotensin II concentrations in shock, but also decreases vascular resistance and, in this way, influences metoprine-induced haemodynamic effects. Interestingly, although there is an increase in angiotensin II plasma concentration after termination of bleeding, neither captopril nor angiotensin receptor antagonists influence haemodynamics in saline-treated animals.

It is an open question, however: are the cardiovascular effects the result of direct vasoconstriction? It is well known that angiotensin II is able to increase

peripheral vascular resistance by releasing catecholamines from peripheral sympathetic nerves (42) and adrenal medullar chromaffin cells (43), potentiating actions of noradrenaline (44) and inhibiting noradrenaline reuptake (45). Therefore, also these indirect mechanisms of angiotensin II action cannot be excluded in metoprine-induced resuscitation.

An increase in plasma angiotensin II concentration and haemodynamic effects of ACE inhibition and AT₁ receptors blockage clearly show central histamine-induced activation of the circulating renin-angiotensin system, however, it is difficult to exclude a possible influence of angiotensin II on the central cardiovascular regulatory mechanisms. Since angiotensin receptors are densely distributed in the circumventricular organs, which have neuronal connections with integrative sites for sympathetic drive, such as the nucleus of the solitary tract (46), in this way circulating angiotensin II can influence autonomic system responses. On the other hand, there is increased release of angiotensin II in the central nervous system in hypovolaemia, which may suggest its involvement in cardiovascular regulation (47). Indeed, angiotensin II sensitises the cardiac sympathetic afferent reflex *via* central AT₁ receptors (48) and increases AVP content in the brain stem cardiovascular regions and thus may potentiate effects of AVP on vagal motor neurones (49). On the other hand, Paczwa *et al.* have shown that upregulation of the brain angiotensinergic system in renin transgenic hypertension is associated with altered function of the vasopressinergic system in haemorrhagic hypovolaemia (50). Therefore, further studies are needed to establish the possible central influence of angiotensin II on metoprine-induced effects.

In conclusion, metoprine leading to an increase in endogenous central histamine concentration produces a long-lasting pressor effect in haemorrhage-shocked rats. The action is accompanied by a rise in angiotensin II plasma concentration, and is diminished by ACE inhibition and AT₁ receptor blockage. These results demonstrate the involvement of the renin-angiotensin system in the resuscitating effect elicited by central endogenous histamine.

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