INTRODUCTION

The central cholinergic system affects the cardiovascular regulation both in normotension and critical hypotension (1-3). Interestingly, there are different cholinergic pathways responsible for blood pressure regulation, depending on the initial status. In normotensive animals the pressor effect of cholinomimetics is short-lasting and mediated through the central muscarinic mechanisms, which leads to the activation of the sympathetic nervous system (1-2). In contrast, in haemorrhage-shocked rats centrally acting cholinomimetic drugs evoke a long-lasting resuscitating action mediated via central nicotinic receptors (3). Similarly to normotension, peripheral mechanisms activated by the central nicotinic pathway in haemorrhagic shock involve the sympathetic nervous system (3).

Cytidine 5'-diphosphocholine (CDP-choline) is an endogenously synthesized mononucleotide which exerts a variety of physiological effects by altering central cholinergic transmission. Administered intracerebroventricularly (i.c.v.) or intravenously, it reverses haemorrhagic hypotension in rats, apparently by the activation of central cholinergic receptors. The study was undertaken to investigate the involvement of the central histaminergic system in CDP-choline-mediated reversal of haemorrhagic hypotension. Experiments were carried out in male ketamine/xylazine-anaesthetised Wistar rats subjected to haemorrhagic hypotension of 20-26 mmHg. CDP-choline (2 µmol; i.c.v.) administered at 5 min of critical hypotension produced a long-lasting pressor effect with increases in mean arterial pressure (MAP), heart rate (HR), and renal, hindquarters and mesenteric blood flows, resulting in a 100% survival at 2 h. The action was accompanied by approximately a 26% increase in extracellular histamine concentration at the posterior hypothalamus, as measured by microdialysis. Cardiovascular effects mediated by CDP-choline were almost completely blocked by pretreatment with H1 receptor antagonist chlorpheniramine (50 nmol; i.c.v.), but not with H2 receptor blocker ranitidine (25 nmol; i.v.) or H3/H4 receptor antagonist thioperamide (50 nmol; i.c.v.). In conclusion, the present results show that the central histaminergic system, through the activation of H1 histaminergic receptors, is involved in CDP-choline-induced resuscitating effect in haemorrhage-shocked rats.

Key words: haemorrhagic shock, histamine, cytosine 5'-diphosphocholine, microdialysis, mean arterial pressure, heart rate
experiments were performed to disclose further interactions between the histaminergic and cholinergic systems. The results were, in part (haemodynamic studies), presented at the XXXVII Annual Meeting of the European Histamine Research Society (Stockholm, Sweden) (13).

MATERIALS AND METHODS

Animals

Studies were performed in male Wistar rats weighing 260-315 g (6-7 months old). The animals were housed in individual cages in the animal colony, under controlled conditions of temperature (20-22°C), humidity (60-70%) and lighting (12 h light/dark cycle), and provided with food and water ad libitum. Each study group consisted of four to nine 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 26/2008).

General procedures

Under ketamine/xylazine (100/10 mg/kg; intramuscularly) anesthesia, the left common carotid artery and the right jugular vein were cannulated with PE 50 tubing filled with heparinized saline (250 U/ml). For intracerebroventricular (i.c.v.) treatment, a burr hole was drilled around the skull 1.6 mm lateral to midline and 0.9 mm posterior to bregma. A 22-gauge stainless steel hypodermic tubing was directed through the hole toward the lateral ventricle. The cannula was lowered 5.0 mm below the surface of the skull and fixed to the skull with acrylic cement. All i.c.v. injections were given in a volume of 5 µl over a period of 60 s.

Cardiovascular parameter measurements

MAP and HR were measured using the pressure transducer RMN-201 (Temed, Zabrze, Poland) and the electrocardiograph Diascope 2 (Unitra Biazet, Bialystok, Poland), respectively. Electromagnetic probes (Type 1RB and 2.5SB, 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). RMN-201 (Temed, Zabrze, Poland) and the electrocardiograph Diascope 2 (Unitra Biazet, Bialystok, Poland), respectively. Electromagnetic probes (Type 1RB and 2.5SB, 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). All measurements of blood flow were started after a 30 min adaptation period to avoid the influence of probe implantation.

Microdialysis study

Handmade microdialysis probes (by Nesrin Filiz) 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 18000 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 NaH₂PO₄, 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 60 min after the start of bleeding. At the end of the experiments, the animals were killed, and for the determination of microdialysis probe localization, brains were fixed in 4% paraformaldehyde, and 50 micrometer-thick vibratom sections were collected. Sections were stained in 1% cresyl violet solution for 15 minutes. 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 DPX. Probe localization was determined and representative pictures were taken using an Olympus BX-50 microscope adapted with a CCD digital camera. Fig. 1 presents the histological verification of microdialysis probe implantation.

Haemorrhagic shock protocol

Irreversible haemorrhagic shock, according to the method of Guarini (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 stabilised at 20-26 mmHg.

Experimental protocol

Our previous studies based on the same haemorrhagic shock model demonstrate that a dose of 2 µmol (i.c.v.) of CDP-choline produces a long-lasting resuscitating effect with a 100% survival at 2 h (7). This dose was, therefore, chosen for present experiments.

After termination of bleeding, separate groups of animals were first given i.c.v. histamine H₁, H₂ and H₁/H₂ receptor antagonists chlorpheniramine (50 nmol), ranitidine (25 nmol) and thioperamide (50 nmol), respectively, or saline (5 µl), and 5 min later – CDP-choline or saline. The doses of histamine receptor blockers were taken from the literature (10, 14). The animals were monitored for 2 h after treatment or until death, if it occurred earlier. Experiments were performed between 9 a.m. and 2 p.m.

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 isotropic system (0.04 mol/l KH₂PO₄, 0.015 mol/l sodium-1-hepta sulfonate 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: CDP-choline (Sigma-Aldrich, USA), chlorpheniramine maleate, ranitidine hydrochloride, thioperamide maleate, xylazine (Research Biochemicals Incorporated, USA), ketamine (Gedeon Richter, Hungary) and heparin (Polfa, Poland). All drug solutions were prepared freshly on the day of the experiment.

Statistics

All values are given as means±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-Keuls. The Fisher’s exact test was used to examine significant differences in survival rates.

RESULTS

The basal pre-bleeding values of MAP, HR and peripheral blood flows did not reveal significant differences between the groups.

The total bleeding volume necessary for the induction of haemorrhagic shock was 2.34±0.31 ml/100 g body weight (n=57). In the control saline-treated group, bleeding from MAP 81.2±7 mmHg to 20-26 mmHg was associated with a decrease in HR from 334±17 beats/min to 212±21 beats/min (n=6). In this group, haemorrhage-induced decreases in RBF (from 5.04±0.66 ml/min to 0.62±0.15 ml/min; n=6), HBF (from 9.51±1.53 ml/min to 1.02±0.34 ml/min; n=6) and MBF (from 7.14±0.63 ml/min to 0.63±0.21 ml/min; n=6).

In the control icv saline-treated group, there were no significant increases in MAP, HR and peripheral blood flows in the post-bleeding period, and all animals died within 30 min.

Influence of CDP-choline on MAP, HR and regional haemodynamics in critically hypotensive rats

In the saline-pre-treated group, CDP-choline induced a long-lasting pressor effect with rises in MAP, HR and peripheral blood flows, which started within 1 min of treatment and lasted until the end of the experiment (Figs. 2, 3). None of the rats died before the end of experiment; the survival rate at 2 h was 100% (P<0.05 vs. the saline pre-treated group; Fisher’s exact test).

Influence of CDP-choline on histamine release in the posterior hypothalamus in critically hypotensive rats

In vivo microdialysis studies showed that the initial extracellular histamine level at the posterior hypothalamus in both groups was 0.17±0.03 pmol/10 min (n=9). Induction of critical haemorrhagic hypotension did not influence histamine concentrations (Fig. 4).

Intracerebroventricular administration of CDP-choline (2 µmol) evoked a 26% increase in extracellular histamine levels at the posterior hypothalamus during the first 10 min after injection (Fig. 4). Similar increases were observed 20, 30 and 40 min after injection. In the control saline-treated group, there were no significant changes in the posterior hypothalamic histamine concentrations (Fig. 4).

Influence of histamine receptor antagonists on CDP-choline-induced resuscitating effect in haemorrhage-shocked rats

Chlorpheniramine almost completely blocked CDP-choline-evoked MAP (Fig. 2A), HR (Fig. 2B) and peripheral haemodynamic changes (Fig. 3). Moreover, it decreased to 0% the survival rate at 2 h (mean survival time 55.1±17.5 min; P<0.05 vs. the saline pre-treated CDP-choline-injected group). In the control saline-treated group, chlorpheniramine did not influence the measured parameters as compared to the saline pre-treated animals (Fig. 2-3).

Neither H2 receptor blocker ranitidine nor H3/H4 receptor antagonist thioperamide influenced cardiovascular and survival rate changes evoked by CDP-choline (Fig. 2-3). Similarly, in the control saline-treated groups, the antagonists did not affect cardiovascular parameters (Fig. 2-3), and the survival rate at 2 h in both groups was 0%.

DISCUSSION

The present results confirm and extend our previous data, demonstrating the resuscitating effect of CDP-choline administered centrally in haemorrhage-shocked rats (7). Moreover, using microdialysis and haemodynamic measurements we provide, for the first time, evidence that the histaminergic system is involved in CDP-choline-mediated cardiovascular action.
As in our earlier studies, we used the pressure-controlled rat model of haemorrhagic shock by Guarini (3), the model characterised by severe irreversible hypotension, early initiation of the sympathoinhibitory phase and development of hypoxaemia and severe metabolic acidosis (15-16). Our present results confirm that in these conditions, CDP-choline evokes the resuscitating effect. It may be of clinical relevance that the action is observed not only after central (i.c.v.) but also peripheral (iv) CDP-choline administration, which is associated with easy penetration of the agent through the blood-brain barrier (6-7).

Previous haemodynamic studies show that CDP-choline-induced increases in MAP and HR are accompanied by a rise in renal and superior mesenteric blood flows as well as an increase in the survival time of haemorrhage-shocked rats (17). Our present results fully confirm those data. The compensatory mechanisms activated by CDP-choline, described so far, include the sympathetic nervous system (6), arginine vasopressin (AVP) (18), ACTH and TSH (19). On the other hand, our previous studies clearly show that histamine, acting centrally as a neurotransmitter, evokes resuscitating action due to the activation of the sympathetic nervous system (20), the renin-angiotensin system (21), and release of AVP (22) and proopiomelanocortin-driven peptides (23). Histamine-mediated
cardiovascular effects in haemorrhagic shock are associated with an increase in circulating blood volume due to its mobilisation from venous reservoirs and rapid compensation of acid-base balance (15, 24).

In the present study, we examined a possible involvement of the histaminergic system in CDP-choline-induced resuscitating effect using cerebral microdialysis and haemodynamic methods. Basal extracellular histamine levels at the posterior hypothalamus were similar to those measured previously by Cenni et al. (0.19±0.03 pmol/15 min) (25). There is a general agreement that histamine, in addition to peptide neuromodulators (26-27), plays a role in the activation of compensatory mechanisms to nociceptive and damaging stimuli and participates in the maintenance of circulatory homeostasis (8). In support, the study by Philippu et al. demonstrates an increase in histamine release from hypothalamic neurons in cats subjected to haemorrhage (28). Interestingly, we revealed that the induction of critical hypotension did not influence histamine concentration, and the discrepancy may be explained by differences in animal species and experimental models.

Our results show for the first time that administration of CDP-choline to haemorrhage-shocked rats leads to a 26% increase in histamine release from the posterior hypothalamic neurons. In our previous study, we have demonstrated the resuscitating action of histamine given i.e.v. at a high dose (100 nmol) in the same haemorrhagic shock model (10). Despite that, we suggest an involvement of the histaminergic system in CDP-choline-induced pressor effect, since the extents of biological actions evoked by different range changes in histamine concentrations in cerebrospinal fluid and extracellular compartment at the posterior hypothalamus are difficult to compare. Although we still do not know whether the stimulating effect of CDP-choline is related to the activation of nicotinic cholinergic receptors directly by choline or indirectly by CDP-choline-derived acetylcholine, our microdialysis data provide evidence that there is a functional relationship between the histaminergic and cholinergic systems. Moreover, our results are in agreement with the studies by Uteshev and Knot, who have shown that the activity of histaminergic neurons can be regulated by cholinergic agents (29).

Haemodynamic studies confirm the involvement of the histaminergic system in CDP-choline-induced resuscitating effect since chlorpheniramine almost completely blocks the increase in MAP, HR and peripheral blood flows. On the other hand, neither ranitidine nor thioperamide affects CDP-choline-mediated cardiovascular changes in haemorrhage-shocked rats. These results are in line with our previous findings, demonstrating the predominant involvement of H1-receptor-mediated pathway in histamine action on the cardiovascular centre in rats (10). Moreover, we have shown previously that chlorpheniramine is able to inhibit the central resuscitating effect elicited by serotonin (30) and 5-HT1A receptor agonist 8-OH-DPAT (31), whereas H1 and H4 receptor antagonists have no significant influence on central histamine-induced resuscitating action (10).

Previously published studies clearly demonstrate interactions between the cholinergic and histaminergic systems regarding central cardiovascular regulation in normotensive rats (32). Interestingly, our recent study provides further evidence that there are functional interactions between the two systems in critical haemorrhagic hypotension (12). We have shown an almost complete blockage of histamine-induced resuscitating action by nicotinic receptor antagonist mecamylamine. In contrast, muscarinic receptor blocker atropine sulphate only partly inhibits MAP and peripheral haemodynamic changes evoked by histamine in haemorrhage-shocked rats (12). These observations have prompted us to suggest that the nicotinic, and not muscarinic, cholinergic pathway is a predominant mechanism of histamine-induced effect in haemorrhagic shock in rats (12). The concept is in agreement with studies demonstrating that histamine is able to activate directly the central cholinergic system (33). The action may be associated with centrally acting prostaglandins (34) which participate in the cholinergic- and adrenergic-mediated stimulation of the hypothalamic-pituitary-adrenal axis – an essential mechanism in the reversal of haemorrhagic hypotension (35-37).

In conclusion, there is a bidirectional interaction between the cholinergic and histaminergic systems in the central cardiovascular regulation of haemorrhagic hypotension in rats. Our results show the activation of the histaminergic neurons

**Fig. 4.** Changes in hypothalamic histamine levels before bleeding, 10 and 20 min after the start of bleeding and 10-40 min after i.e.v. CDP-choline or saline injections; initial extracellular histamine level in animals before bleeding is 0.17±0.03 pmol/10 min (n=9); *P < 0.05 vs. the post-bleeding value, #P < 0.05 vs. saline-treated (control) group. Note that there are no results of histamine levels in the control group at 30 and 40 min, because all animals died before the collections.
and the involvement of H1 receptor-mediated neuronal pathway in CDP-choline-induced resuscitating effect.

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REFERENCES

32. Mlynarska MS. Interaction between the central histaminergic and the muscarinic cholinergic systems. Agents Actions 1994; 41: C82-C84.

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