INTRODUCTION

Histaminergic neurones concentrated in the tuberomammillary nucleus of the hypothalamus influence many central nervous system functions, including energy balance, drinking, pain perception, learning and memory as well as cardiovascular and respiratory regulations (1). Histamine, acting as a neurotransmitter, influences the central cardiovascular regulation both in normotensive (2) and critically hypotensive animals (3). Inhibition of histamine N-methyltransferase (HNMT; EC 2.1.1.8) activity with SKF 91488, leading to an increase in endogenous central histamine concentrations due to the blockage of its catabolism evokes a dose-dependent pressor effect in normotensive rats (4). Similar action is observed after administration of exogenous histamine into the brain lateral ventricle (icv) (2).

Our previous studies demonstrate that histamine, acting centrally as a neurotransmitter, evokes a reversal of haemorrhagic shock in rats due to the activation of the sympathetic and the renin-angiotensin systems as well as the release of arginine vasopressin and proopiomelanocortin-derived peptides. In the present study, we demonstrate influences of cholinergic receptor antagonists on the central histamine-induced resuscitating action. Experiments were carried out in male anaesthetised Wistar rats subjected to a haemorrhagic hypotension of 20-25 mmHg, resulting in the death of all control animals within 30 min. Histamine (100 nmol) administered intracerebroventricularly (icv) at 5 min of critical hypotension produced a long-lasting pressor effect with increases in heart rate and peripheral blood flows, and a 100% survival at 2 h. Mean arterial pressure and blood flow changes were almost completely blocked by nicotinic receptor antagonist mecamylamine (246.3 nmol; icv) and partially inhibited by muscarinic receptor blocker atropine sulphate (14.8 nmol; icv). Cholinergic receptor antagonists given alone in the control saline-treated groups did not affect cardiovascular parameters in the post-bleeding period. In conclusion, there are interactions between the histaminergic and cholinergic systems, with an involvement of both nicotinic and muscarinic receptors, in the central cardiovascular regulation in haemorrhagic hypotension in rats.

Key words: haemorrhagic shock, histamine, cholinergic system, rats

MATERIALS AND METHODS

Animals

Studies were carried out in male Wistar rats weighing 265-450 g (6-9 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 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).
Surgical preparations

For icv treatment, the rats were implanted, under ketamine/xylazine (100 mg/kg/10 mg/kg; ip) anaesthesia, of polyethylene cannula into the right brain lateral ventricle (3). All icv injections were made in a volume of 5 µl over a period of 60 s. Anaesthetised rats were implanted with catheters filled with heparinized saline (100 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 Bielat, 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) (5). 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. (16), 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

Since our previous studies performed on the same haemorrhagic shock model demonstrate that a dose of 100 nmol (icv) of histamine produces a long-lasting pressor effect with a 100% survival at 2 h (3), that dose was chosen for the present experiments.

To examine the involvement of the cholinergic system in histamine-induced reversal of haemorrhagic shock, histamine was injected 5 min after termination of bleeding, after icv pre-treatment with muscarinic receptor antagonist atropine sulphate (14.8 nmol), nicotinic receptor blocker mecamylamine (246.3 nmol) or saline (5 µl). In the control groups, the rats were pre-treated with cholinergic receptor antagonist atropine sulphate (14.8 nmol), mecamylamine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA), xylazine hydrochloride (Research Biochemicals Inc., Natick, MA, USA), ketamine (Gedeon Richter, Budapest, Hungary), heparin (Polfa, Warszawa, Poland). All drug solutions were prepared freshly on the day of the experiment.

Drugs

The following drugs were used: atropine sulphate, histamine dihydrochloride, mecamylamine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA), xylazine hydrochloride (Research Biochemicals Inc., Natick, MA, USA), ketamine (Gedeon Richter, Budapest, Hungary), heparin (Polfa, Warszawa, 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 initial 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 regional vascular resistance.

The total bleeding volume for the induction of haemorrhagic shock was 2.44 ± 0.18 ml/100 g body weight. In the control group, bleeding from MAP 82.6 ± 5.82 mmHg to 20-26 mmHg was associated with a decrease in HR from 345 ± 17 beats/min to 224 ± 28 beats/min. Haemorrhage induced decreases in RBF from 6.79 ± 0.82 ml/min to 0.52 ± 0.16 ml/min, HBF from 14.8 ± 2.38 ml/min to 1.58 ± 0.49 ml/min and MBF from 8.15 ± 0.86 ml/min to 0.76 ± 0.33 ml/min.

In the control 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 atropine sulphate and mecamylamine on MAP, HR and regional haemodynamic effects of histamine in critically hypotensive rats

In the saline pre-treated group, histamine evoked a long-lasting pressor effect with rises in MAP, HR and peripheral blood flows, which started within 1 min after treatment and
Inhibition of histamine-induced increase in MAP (Fig. 1A) and peripheral blood flows (Fig. 2) showed a significant decrease in the control saline-treated group. The survival rate at 2 h in the saline-pre-treated group was 100% (P < 0.05 vs. the control saline-treated group) which is significantly higher than in the control saline-treated group. In the control saline-treated group, a decrease in peripheral resistance and a rise in blood pressure demonstrated that histamine-activated compensatory mechanisms evoke over a 100% increase of circulating blood volume 20 min after treatment (21), probably due to a mobilisation of blood from venous reservoirs. Thus, an increase in circulating blood volume, possibly together with a lowered responsiveness to endogenous vasoconstrictive factors characteristic for a late phase of shock (22), seems to be responsible for a decrease in peripheral resistance and rises in regional blood flows in central histamine-induced resuscitation.

The cholinergic system influences the cardiovascular centre function both in normotension and critical hypotension; however, there are different cholinergic pathways responsible for blood pressure regulation in normotensive and critically-hypotensive animals (23-24).

In normotensive animals, the hypertensive effect of cholinomimetics is short-lasting and mediated through the central muscarinic mechanisms, which leads to the activation of the sympathetic nervous system (23-24). In contrast, studies by Guarini et al. (16) which show that centrally-mediated resuscitating action of cholinomimetic drugs is long-lasting and mediated via central nicotinic receptors, since it is prevented by nicotinic receptor antagonist mecamylamine, but not by muscarinic receptor blockers atropine, pirenzepine and 4-DAMP (16). Peripheral mechanisms activated by central nicotinic receptors in haemorrhagic shock involve the sympathetic nervous system, similarly as in normotensive animals (16).

Our present results demonstrate that atropine sulphate partially inhibits MAP and peripheral haemodynamic changes evoked by histamine in haemorrhage-shocked rats. Similarly, a blockage of central muscarinic receptors diminishes the pressor effect of histamine injected icv in normotensive rats (23). On the other hand, an influence of the cholinergic muscarinic pathway in the recovery from haemorrhagic shock has previously been reported regarding to POMC-derived peptides (25) and TRH (26). In ACTH-induced reversal of haemorrhagic shock, hemicholinium-3 which causes depletion of brain neuronal acetylcholine prevents the effect (25). Similarly, atropine almost completely inhibits the resuscitating action elicited by given alone did not produce cardiovascular effects in comparison to saline-treated group (Fig. 1, 2).

Mecamylamine almost completely blocked histamine-induced MAP (Fig. 1A) and peripheral haemodynamic changes (Fig. 2), with no influence on HR (Fig. 1B), and decreased to 0% the survival rate at 2 h (mean survival time 47.6 ± 25.4 min; P < 0.05 vs. the saline pre-treated histamine-injected group). In the control saline-treated groups, mecamylamine did not influence significantly measured parameters in comparison to the saline pre-treated animals (Fig. 1, 2).

**DISCUSSION**

The present results are in agreement with our previous studies showing that the activation of the central histaminergic system leads to the resuscitating effect in a rat model of haemorrhagic shock (5-8). Moreover, we demonstrate for the first time that blockage of the central muscarinic and nicotinic receptors inhibits histamine-induced cardiovascular effects in haemorrhage-shocked rats.

In the present study, we used the pressure-controlled model of haemorrhagic shock by Guarini et al. (16) which is characterised by severe irreversible hypotension (MAP 20-26 mmHg), an early initiation of the sympathoinhibitory phase with bradycardia as well as development of hypoxemia and severe metabolic acidosis (20). Our present results confirm that in these conditions, histamine acting centrally produces the resuscitating effect. Previous haemodynamic studies based on the same model of shock demonstrate that histamine-activated compensatory mechanisms evoke over a 100% increase of circulating blood volume 20 min after treatment (21), probably due to a mobilisation of blood from venous reservoirs. Thus, an increase in circulating blood volume, possibly together with a lowered responsiveness to endogenous vasoconstrictive factors characteristic for a late phase of shock (22), seems to be responsible for a decrease in peripheral resistance and rises in regional blood flows in central histamine-induced resuscitation.

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intravenous injection of ACTH-(1-24) (27) and central M₃ muscarinic receptors are involved (28). Thus, Bertolini \textit{et al.} propose two distinct cholinergic pathways activated in cholinomimetics- and ACTH-induced resuscitation, mediated by different types of receptors - muscarinic subtype in the case of ACTH and nicotinic in the case of cholinergic drugs (29). In contrast, the study by Onat \textit{et al.} presents the pressor effect with a decrease in mortality elicited by muscarinic receptor agonist oxotremorine administered to haemorrhage-shocked rats, and the effects are mediated via central muscarinic and nicotinic receptors (30).

ACTH-(1-24)-induced resuscitating effect is mediated via central melanocortin M₄ receptors (31), which triggers the activation of recently identified cholinergic anti-inflammatory pathway with subsequent suppression of the transcription of nuclear factor κB (NF-κB) and activation of inflammatory cascade (32). The mechanism involves central muscarinic and peripheral nicotinic receptors (32). Interestingly, recent data suggest that also in normotensive rats, a major part of the carbachol-induced ACTH and corticosterone secretion results from peripheral cholinergic muscarinic receptor stimulation (33). Our previous study show that endogenous central histamine-induced resuscitating action associated with an increase in plasma ACTH and α-MSH concentrations (8). Moreover, the role of MC₄ receptors in the effect has been presented since a selective MC₄ receptor antagonist HS014 inhibits MAP changes evoked by histamine (8). Therefore, since our present results demonstrate an involvement of muscarinic receptors in histamine-induced resuscitating effect, we propose that also histamine - indirectly, via POMC-derived peptides secretion - could activate cholinergic anti-inflammatory pathway in haemorrhagic shock.

Our present studies show almost a complete blockage of histamine resuscitating action by mecamylamine. In contrast, the study by Mlynarska shows an involvement of cholinergic muscarinic pathway in central histamine-induced cardiovascular effects in normotensive rats (34). The difference can be explained by distinct central cholinergic pathways involved in the cardiovascular regulation in haemorrhaged and normovolaemic rats (16, 23). It has been suggested that a decrease in central cholinergic neurones activity results in hypotension and cardiovascular decompensation which are characteristic for shock conditions (23). On the other hand, histamine is able to activate directly the central cholinergic system (14, 15), and its action may be associated with centrally acting prostaglandins (35) which participate in the cholinergic- and adrenergic-mediated stimulation of the hypothalamic-pituitary-adrenal axis (36, 37). Our recent studies confirm the involvement of the nicotinic mechanism in cytidine-5′-diphosphate choline (CDP-choline)-mediated resuscitating effect in conscious rats (38, 39), which is probably related to a higher supplementation of cholinergic neurones with choline. According to our present results, we suggest that the nicotinic pathway is also a predominant mechanism of histamine-induced effect in haemorrhagic shock in rats.

In conclusion, present results demonstrate the involvement of the nicotinic and muscarinic mechanisms in the central histamine-induced reversal of haemorrhagic shock in rats.

Conflict of interests: None declared.

REFERENCES


19. Savci V, Goktalay G, Cansev M, Cavus S, Yilmaz MS, Ulus IH. Intravenously injected CDP-choline increases blood


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


Received: August 21, 2009
Accepted: April 30, 2009

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