INTRODUCTION

Haemorrhagic shock is a state of circulatory disorder caused by critically low blood volume induced by excessive blood loss. In physiological conditions the blood volume is controlled by several regulatory mechanisms, including vasopressin, natriuretic peptides, renin-angiotensin-aldosteron axis and corticosteroids. Furthermore, water income and elimination also plays an important role (1).

According to American College of Surgeons and modified by Gutierrez et al., haemorrhage may be divided into four groups depending on the blood loss volume: class I <15%, class II 15–30%, class III 30–40% and class IV >40% blood loss of total blood volume (2). Haemorrhagic shock is usually achieved after the loss of 35–40% of total blood (class III or IV).

In 1991 Schadt and Ludbrook hypothesized that the response of mammals (including humans) to progressive acute hypovolaemia is double-phased (3). During the first phase, the decrease in blood volume induces several compensatory neurohormonal mechanisms that help maintain the blood pressure at almost normal values (1, 4, 5). These mechanisms include an increase in sympathetic outflow and decrease in vagal nerves activity resulting in serum noradrenaline increase, increase in heart rate and peripheral vasoconstriction. This phase is called the sympathoexcitatory phase. After further bleeding and the loss of approximately 35–50% of total blood volume the second sympathoinhibitory phase is about to reveal (3). There is a rapid decrease in blood pressure and heart rate as a result of a paradoxical stimulation of the left heart ventricle mechanoreceptors initiating the Bezold-Jarisch reflex (6). The parasympathetic nervous system is stimulated and sympathetic neurons are inhibited, except the adrenal sympathetic fibers (further epinephrine release) (7). Furthermore, the activity of the renin-angiotensin-aldosteron system, serum vasopressin and proopiomelanocortin are increased (8, 9). Nevertheless, the blood pressure dramatically decreases.

The bleeding and haemorrhage is strictly related with accidents and many medical procedures. In some conditions it leads to hypovolaemia and further to hypovolaemic shock. Under conditions of haemorrhagic shock, heart rate and blood pressure critically collapse. Reversing the sympathoinhibitory phase of hypovolaemia could be crucial for clinical management of injured patients after haemorrhage. Systemic administration of 5-HT1A agonists seams to produce resuscitating effects. The aim of this study was to investigate the participation of central serotonin and, in particular, 5-HT1A receptors in cardiovascular regulation in haemorrhagic shock in rats. Intracerebroventricular (i.c.v.) administration of serotonin (5-HT) increased the heart rate (HR), mean arterial pressure (MAP) and implicated that all haemorrhaged animals survived for the whole observation time (2 hours). Similar, although significantly more minor, effects were achieved after selective 5-HT1A activation. Moreover, the i.c.v. administration of selective 5-HT1A antagonist before i.c.v. 5-HT injection partially inhibited 5-HT induced changes. The results of the present work indicate that 5-HT plays an important role in the reversal of the haemorrhagic shock in rats. These effects are at least partially dependent on activation of 5-HT1A receptors.

Key words: serotonin, 5-HT1A receptors, haemorrhagic shock, cardiovascular system, mean arterial pressure, heart rate
that peripheral administration of 5-HT influenced the blood pressure in a 3-phase response (decrease/increase/decrease) (21) and modulated vagal nerves activity (22).

Centrally administered 5-HT in normotensive animals induced hypotensive effect with bradycardia as a result of 5-HT1A activation located in rostral ventrolateral medulla (15, 16). Moreover it inhibits HR and MAP changes after activation of dorsomedial hypothalamic nucleus (23). Other articles deny the influence of 5-HT1A in cardiovascular regulation (17). Inhibition of 5-HT1A using NAN-190 did not reveal any changes in HR, MAP or peripheral blood flow in normotensive rats (24).

In the recent years the team of Scrogin et al. performed a several research on 5-HT actions in cardiovascular control during hypovolaemia. It was revealed that, buspirone (partial 5-HT1A agonist) given systemically, restored blood pressure and HR in hypotensive phase of haemorrhage in conscious rats. Those effects were dose dependent (25). Resuscitating effects were strictly related to the rise in endogenous sympathetic drive resulting in increase in cardiac output (26) and mobilizing existing blood stores (27). Moreover it was shown, that spleen plays a crucial role in response to 8-OH-DPAT activation of sympathetic system (28). In previous research there is a lack of information on centrally acting 5-HT.

The aim of the study was to investigate hemodynamic effects caused by the intracerebroventricular injection of exogenous serotonin, particularly the participation of 5-HT1A receptors during haemorrhagic shock in rats.

MATERIAL AND METHODS

The study was conducted on Wistar male rats, weighing in the range of 210–280 g. The animals were kept in metal cages, five per cage, under the 12-hour day/night cycle, water and food ad libitum. All experimental procedures were carried out under full anesthesia. The lateral ventricle cannulation was performed at 5–7 days before the main experiment, using ketamine (100 mg/kg) (Gedeon Richter, Budapest, Hungary) and xylazine (10 mg/kg) (Research Biochemicals Inc., Natick, MA, USA) given intraperitoneally (i.p.). The 8 mm diameter cannule, after skin preparation, was placed in the skull according to Hilliard et al. (29), deep on 4.4.5 mm, and fixed with acrylic glue. All substances administered intracerebroventricularly (i.c.v.) were in the volume of 5 µl using a Hamilton syringe (type Microliter #702, Hamilton-Bonaduz, Switzerland).

On the day of the main experiment the animals were anesthetized using ethylurethane (Riedel-de Haen, Seelze, Germany) (1.25 g/kg; i.p.). After superficial tissue preparation, femoral artery and femoral vein were cannulated with catheters filled with heparinized 0.9% NaCl (300 IU/ml) (Polfa, Warsaw,

Fig. 1. Changes in HR (A) and MAP (B) after haemorrhagic shock induction and i.c.v. 5-HT administration in the doses of 50 µg (■), 100 µg (▲) and in control group (○). The moment of i.c.v. 5-HT solutions or 0.9% saline solution injection was marked by the arrow. The values were presented as mean ±standard deviation (S.D.), n=6, * p<0.05 vs. control group.
Poland). In animals in which the peripheral blood flow was measured, the carotid communal artery and internal jugular vein were cannuled. For the peripheral blood flow measurement, after previous laparotomy, the renal artery, upper mesenteric artery and the distal part of abdominal aorta were prepared.

Systolic, diastolic and mean arterial blood pressure (MAP) was measured using RMN-201 (Temed, Zabrze, Poland). Heart rate (HR) was automatically calculated as QRS rate in electrocardiography diascope 2 (Unitra Biazet, Białystok, Poland). Peripheral blood pressure were measured using electromagnetic sensors (1RB2006, Hugo Sachs Elektronik, March-Hugstetten, Germany) connected to time flowmeter type 700 (Hugo Sachs Elektronik, March-Hugstetten, Germany).

Peripheral resistance was calculated as a ratio of MAP to peripheral flow in renal artery (RBF), upper mesenteric artery (MBF) and the distal part of abdominal aorta (hindlimb blood flow, HBF).

The principal experiment included a 2 hour observation period, after cardiovascular parameters stabilization in haemorrhagic shock.

Haemorrhagic shock was evoked according to Guarini et al. (30-32), by controlled bleeding from the femoral or internal jugular vein to a calibrated drain with maximal speed of 1 ml/min, over the period of 15–25 min, reaching and stabilizing of MAP between 20-25 mmHg.

To examine the influence of central serotonin 1A receptor (5-HT1A) in cardiovascular regulation under haemorrhagic shock conditions, selective 5-HT1A antagonist - NAN-190 (Research Biochemicals Inc., Natick, MA, USA) (10 µg/5 µl) and selective 5-HT1A agonist - 8-OH-DPAT (Riedel-de Haen, Seelze, Germany) (5 µg/5 µl) were used i.c.v. NAN-190 was administered 5 min. before i.c.v. serotonin and 8-OH-DPAT application.

The amount of given 5-HT1A ligands were selected according to the preliminary study. We used 8-OH-DPAT in four concentrations 1, 3, 5 and 7 µg, choosing the concentration of 5 µg because of its optimal effects (survival of all tested animals). In lower concentrations 8-OH-DPAT did not secure 100% survival. Moreover the results of preliminary study revealed no statistical difference between the dose of 5 and 7 µg (data not shown). Similar amounts were used by previous authors (33). The concentration of NAN-190 was chosen after the previous experiments performed in our department confirmed by our preliminary experiments (34).

According to the fact that serotonin is an unstable substance, in the present study, serotonin creatinine sulfate complex was used (35) (Riedel-de Haen, Seelze, Germany).

Statistical analysis was performed using Statistica 7.0 (StatSoft Inc, OK, USA). The parametric t-Student test and non-parametric U-Man-Whitney, $\chi^2$ and Wilcoxon tests were used for

![Fig 2](image-url) Changes in HR (A) and MAP (B) after i.c.v. 50 µg of 5-HT administration (■) and i.c.v. 10 µg of NAN-190 premedication (▲) and in control groups in which 0.9% saline (◇) or NAN-190 without 5-HT (○) were used. The moment of i.c.v. premedication with 0.9% saline or NAN-190 was marked with the dashed arrow, while the moment of 5-HT injection or 0.9% saline administration in controls was marked as the solid arrow.

The values were presented as mean ± standard deviation (S.D.), n=6, * p<0.05 vs. control group, # p<0.05 vs. 5-HT only treated group.
significance analysis. The values were presented as mean ±standard deviation (S.D.). As statistically significant p<0.05 were accepted.

Experimental protocol was accepted by the local Ethical Committee of Silesian Medical University in Katowice (N° 46/05, 26th of July 2005).

RESULTS

Initial values of heart rate (HR) and mean arterial blood pressure (MAP) in control animals were 302 ±11 beats/min and 80.2 ±4.8 mmHg respectively and there were no significant changes between the observed groups. An average blood volume

![Fig. 3. Peripheral blood flow RBF (A), MBF (B) and HBF (C) changes after i.c.v. administration of 50 µg of 5-HT (■) and i.c.v. 10 µg of NAN-190 (▲) premedication or in control groups in which 0.9% saline (△) or NAN-190 without 5-HT (◇) were used. The moment of i.c.v. premedication with 0.9% saline or NAN-190 was marked with the dashed arrow, while the moment of 5-HT injection or 0.9% saline administration in controls was marked as the solid arrow. The values were calculated as percent of initial values and presented as mean ±standard deviation (S.D.), n=6, * p<0.05 vs. control group, # p<0.05 vs. 5-HT only treated group. Initial values in controls treated with 0.9% saline: RBF 5.82±0.97 ml/min, MBF 7.59±1.75 ml/min, HBF 8.94±1.74 ml/min.]
required for indicating critical hypovolaemia was about 2.18 ±0.25 ml/100 g body mass. After 5 min from achieving haemorrhagic shock, the HR and MAP values in all groups were similar and, in the control group, reached 185 ±12 beats/min and 22.3 ±1.8 mmHg, respectively.

Moreover, mean values of peripheral blood flow - renal blood flow (RBF), mesenteric blood flow (MBF) and hindlimb blood flow (HBF) were statistically similar in all groups and decreased after bleeding to approximately 9–15% of initial values in all animals.

In the control group (after ivc administration of 0.9% NaCl solution) there were no significant changes in the HR and MAP and those animals died before the 30 min. mark of haemorrhagic shock observation.

**Fig. 4.** Peripheral resistance RVR (A), MVR (B) and HVR (C) changes after i.c.v. administration of 50 µg of 5-HT (●) and i.c.v. 10 µg of NAN-190 (▲) premedication or in control groups in which 0.9% saline (◇) or NAN-190 without 5-HT (○) were used. The moment of i.c.v. premedication with 0.9% saline or NAN-190 was marked with the dashed arrow, while the moment of 5-HT injection or 0.9% saline administration in controls was marked as the solid arrow.

The values were calculated as percent of initial values and presented as mean ±standard deviation (S.D.), n=6, * p<0.05 vs. control group, # p<0.05 vs. 5-HT only treated group. Initial values in controls treated with 0.9% saline: RVR 13.78±4.11 mmHg/ml/min, MVR 10.57±3.15 mmHg/ml/min, HVR 8.97±2.04 mmHg/ml/min.
Influence of i.c.v. 5-HT administration on HR and MAP variations in haemorrhage shocked rats.

In critically haemorrhaged rats (MAP about 20–25 mmHg) 5-HT was administered i.c.v. in three doses: 25, 50 and 100 µg. In animals receiving 25 µg of 5-HT there was a significant increase in the HR (Fig. 1A) and MAP (Fig. 1B) compared to controls (p<0.05) and the 2 hour survival rate reached 100%. Because all animals survived in groups where 50 µg or 100 µg of 5-HT were used, the lower dose (50 µg) was chosen for further investigation.

Influence of selective 5-HT1A receptor antagonist NAN-190 on variations of HR, MAP, peripheral resistance and peripheral blood flow induced by centrally administered serotonin.

Selective 5-HT1A receptor antagonist NAN-190 was administered icv in the dose of 10 µg 5 min after bleeding and 5 min before i.c.v. 5-HT administration.

Central NAN-190 premedication caused partial inhibition of HR (Fig. 2A) and MAP (Fig. 2B) changes induced by centrally acting serotonin (p<0.05). NAN-190 had no influence on the survival rate.

NAN-190 given alone (without serotonin) did not influence the circulatory parameters (Fig. 2, Fig. 3, Fig. 4).

Peripheral resistances: renal vascular resistance (RVR) (Fig. 4A), mesenteric vascular resistance (MVR) (Fig. 4B) and hindlimb vascular resistance (HVR) (Fig. 4C) were significantly lower after NAN-190 administration. The peripheral resistance decrease was accompanied by HBF increase in 90 and 120 min of observation (Fig. 3C), while RBF (Fig. 3A) and MBF (Fig. 3B) revealed no significant changes compared to the animals without the 5-HT1A blockade.

Influence of centrally administered selective 5-HT1A agonist - 8-OH-DPAT on HR, MAP, peripheral resistance and peripheral blood flow in haemorrhage shocked rats.

Central administration of selective 5-HT1A agonist 8-OH-DPAT (5 µg, i.c.v.) caused a significant increase in the HR (Fig. 5A) and MAP (Fig. 5B) compared to the controls and increased the survival rate to 100%. The observed increase in the HR and MAP was similar although statistically lower compared to those observed after the 5-HT treatment. Moreover, HR (Fig. 5A) changes revealed later compared to i.c.v. serotonin treated animals.

Peripheral vascular resistances: RVR (Fig. 7A), MVR (Fig. 7B) and HVR (Fig. 7C) were significantly lower after 8-OH-DPAT administration compared to 5-HT treated group while RBF (Fig. 6A), MBF (Fig. 6B) and HBF (Fig. 6C) did not differ between these groups.

Simultanous treatment of centrally administered selective 5-HT1A agonist - 8-OH-DPAT and selective 5-HT1A receptor antagonist NAN-190 in haemorrhage shocked rats.

**Fig. 5.** Changes in HR (A) and MAP (B) after i.c.v. 8-OH-DPAT administration in the dose of 5 µg (●) compared with i.c.v. administration of 50 µg of 5-HT (■) and in control group, where only 0.9% saline was used (○). The moment of i.c.v. premedication with 0.9% saline was marked with the dashed arrow, while the moment of 8-OH-DPAT, 5-HT or 0.9% saline administration was marked as the solid arrow.

The values were presented as mean ±standard deviation (S.D.), n=6, * p<0.05 vs. control group, # p<0.05 vs. 5-HT treated group.
In our experiment, i.c.v. premedication with NAN-190 totally blocked the resuscitative effects of 8-OH-DPAT in hypovolaemic shock in rats. All tested animals died between 15–30 min. of hypovolaemia and the results did not differ compared to control group (data not shown).

**DISCUSSION**

In our study, the mean blood volume loss necessary for achieving the required MAP (20–25 mmHg) was about 2.18±0.25 ml/100 g body mass, i.e., about 40–45% of total blood.

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Fig. 6. Peripheral blood flow RBF (A), MBF (B) and HBF (C) changes after i.c.v. administration of 5 µg of 8-OH-DPAT (●) compared with i.c.v. injection of 50 µg of 5-HT (■) and in control group, where only 0.9% saline was used (○). The moment of i.c.v. premedication with 0.9% saline was marked with the dashed arrow, while the moment of 8-OH-DPAT, 5-HT or 0.9% saline administration was marked as the solid arrow.

The values were calculated as percent of initial values and presented as mean ± standard deviation (S.D.), n=6, * p<0.05 vs. control group, # p<0.05 vs. 5-HT only treated group. Initial values in controls treated with 0.9% saline: RBF 5.82±0.97 ml/min, MBF 7.59±1.75 ml/min, HBF 8.94±1.74 ml/min.
volume, resulting in HR decrease to about 185±12 vs. 302±11 beats/min (p<0.05) and MAP decrease to average 22.3±1.8 vs. 80.2±4.8 mmHg (p<0.05). The results were similar to previously reported (31, 32, 36). These circulatory parameters variations were due to the sympathetic system activity inhibition following the Bezol-Jarisch reflex from the left heart ventricle receptors. Blood loss induces compensatory mechanisms activation including blood vessels constriction and peripheral resistance increase. In our study, the peripheral blood flow RBF, MBF and HBF 5 min. after the initiation of bleeding achieved about 10% of initial values and peripheral resistance increased to average 250–300% compared to those before bleeding.

Fig. 7. Peripheral resistance RVR (A), MVR (B) and HVR (C) changes after i.c.v. administration of 5 µg of 8-OH-DPAT (●) compared with i.c.v. injection of 50 µg of 5-HT (■) and in control group, where only 0.9% saline was used (○). The moment of i.c.v. premedication with 0.9% saline was marked with the dashed arrow, while the moment of 8-OH-DPAT, 5-HT or 0.9% saline administration was marked as the solid arrow. The values were calculated as percent of initial values and presented as mean ± standard deviation (S.D.), n=6, * p<0.05 vs. control group, # p<0.05 vs. 5-HT only treated group. Initial values in controls treated with 0.9% saline: RVR 13.78±4.11 mmHg/ml/min, MVR 10.57±3.15 mmHg/ml/min, HVR 8.97±2.04 mmHg/ml/min.
Most authors confirm the influence of centrally given 5-HT on cardiovascular regulation, although its functions remain unknown. There is still a lack of information on this topic and very few of them concern the subject under hypovolaemic conditions. Previous findings are diversified and even contradictory (37-39).

In our study, the i.c.v. administration of 5-HT in rats partially reversed the sympathoinhibitory phase of haemorrhagic shock with HR, MAP, RBF, MBF and HBF increase. Moreover, 2 hour survival rate increased from about 50% to 100%. These resuscitative effects of 5-HT were dose-dependent. The most effective dose of 50 µg and 100 µg caused a 100% survival rate with HR 278±12 and 289±23 beats/min and MAP 52.8±5.9 and 52.6±8.6 mmHg, respectively, after 120 min of observation.

These findings suggest an important role of centrally acting 5-HT to reverse the sympathoinhibitory phase of haemorrhagic shock in rats. In the early 90’s of the XXth century, Evans et al. reported that methysergide, 5-HT1A/5-HT2 receptor non-selective antagonist with partial agonistic effect on those receptors, administered centrally to the fourth ventricle, lateral ventricle and pontomedullary cistern, or given peripherally, delayed the development of haemodynamic effects evoked by 8-OH-DPAT. The activation of the 5-HT1A receptors seems to play the crucial role in actions of that agonist in those precise conditions.

Additionally, we investigated the role of central 5-HT1A receptors in the resuscitative effect of 5-HT. We used a selective antagonist (NAN-190) and agonist (8-OH-DPAT) of 5-HT1A receptors. Premedication of NAN-190 given i.c.v. 5 min before i.c.v. 5-HT administration caused partial attenuation of the HR and MAP increase caused by 5-HT. Moreover, peripheral resistance was significantly lower compared to 5-HT only treated animals. The peripheral blood flow was mainly similar in both groups (except for HBF in 90 and 120 min), probably because of critically low blood volume. In animals treated with 8-OH-DPAT 5 min after reaching hypovolaemic shock, we observed 100% survival for 120 min with accompanying HR, MAP and peripheral blood flow increase. The haemodynamic effects evoked by 8-OH-DPAT were similar, but significantly less pronounced compared to those of 5-HT developed. Premedication with NAN-190 totally blocked resuscitative effects of 8-OH-DPAT.

Present results evidence the participation of central 5-HT1A receptors in the resuscitative effect of 5-HT in haemorrhage shocked rats. Some previous reports indicate such an effect of those receptors (37, 41) while others suggest promoting shock impact (40, 42). The difference between the outcome of our study and previous reports may result from different experimental protocols, altitude of hypotension or the form of 5-HT used. Moreover, our results support previously published results from Titiakov and Scogrin (26, 27), using similar drugs but different protocols.

Some reports suggest the influence of other 5-HT receptors, i.e., 5-HT2, 5-HT3 or 5-HT7 in circulatory regulation. Microinjections of DOI - 5-HT2 agonist, into the region of the paraventricular nucleus in rats induced HR and MAP increase (17). Bazzani et al. reported inhibition of the resuscitative effect of ACTH by the blocking of 5-HT2 and 5-HT3 receptors in haemorrhage shocked rats (43). Moreover some findings revealed 5-HT7 receptors participation in vagal-related regulation of HR (44).

The facts, observed in our study, that NAN-190 only partially attenuated 5-HT related resuscitation, and that 8-OH-DPAT influences haemodynamic parameters to a minor degree compared with 5-HT, confirms the participation of other 5-HT receptors in cardiovascular regulation in haemorrhagic shock in rats. It seems clear that central 5-HT regulation of circulation is multi-receptoral.

Moreover, although 8-OH-DPAT was used as agonist of 5-HT1A receptors, its activation of 5-HT1A receptors needs to be pointed out and, as such, further investigation of this very phenomenon is required (45). However, because the use of NAN-190 totally blocked the effects of 8-OH-DPAT, the activation of the 5-HT1A receptors seems to play the crucial role in actions of that agonist in those precise conditions.

There are also some reports that effects of 5-HT on cardiovascular system partially depends on histaminergic system (34, 46). Serotonin receptors may also be involved in hypotensive effects induced by antidepressant drugs (47). The present work brings new data concerning the influence of centrally acting serotonin, particularly 5-HT1A receptors and gives additional records for better understanding of haemorrhagic shock pathophysiology, which can have clinical implications in the future.

In conclusion, the results of this study indicate that serotonin participates in the central regulation of cardiovascular system in haemorrhage shocked rats. The activation of central serotonergic system induces resuscitative effects including increase in HR and MAP, and takes part in controls of vascular resistance and regional blood flow. These effects are at least partially dependent on the central 5-HT1A receptors.

Conflict of interests: None declared.

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