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PRACA ORYGINALNA
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Involvement of central histaminergic system in cardiovascular effects of Y_1 receptor antagonist BIBP 3226 in haemorrhagic shock in rats

Udział ośrodkowego układu histaminergicznego w efektach krążeniowych antagonisty receptorów Y_1 we wstrząsie krwotocznym u szczurów

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ABSTRACT

INTRODUCTION: Activation of the central histaminergic system leads to the reversal of experimental haemorrhagic shock, whereas neuropeptide Y (NPY) administered intracerebroventricularly (icv) induces a depressor effect in haemorrhagic hypotension. Since histaminergic neurons of the tuberomammillary nucleus receive input from neurons producing NPY localized in the caudal magnocellular nucleus of the hypothalamus, the aim of the study was to examine (1) the cardiovascular effects of the NPY type 1 (Y_1) receptor antagonist in haemorrhagic shock and (2) the possible involvement of the histaminergic system in this action.

MATERIAL AND METHODS: Experiments were performed in ketamine/xylazine-anaesthetised male Wistar rats subjected to irreversible haemorrhagic hypotension, with mean arterial pressure (MAP) 20–25 mmHg and 0% survival at 2 h. Immediately after terminating bleeding, the animals were pre-treated icv with histamine H_1 and H_2 receptor antagonists chlorpheniramine (50 nmol) and ranitidine (50 nmol) as well as the $H_{3/4}$ receptor antagonist/inverse agonist thioperamide (50 nmol), respectively, or saline. Five minutes later, the rats were injected icv with the Y_1 receptor antagonist BIBP 3226 (64 nmol/kg).

RESULTS: BIBP 3226 evoked rises in MAP, pulse pressure and renal blood flow (RBF) with an increase in survival to 100% at 2 h. Chlorpheniramine inhibited cardiovascular changes evoked by BIBP 3226 and decreased to 0% survival at 2 h. In contrast, ranitidine and thioperamide had no effect.

CONCLUSIONS: We demonstrate for the first time (1) the pressor effect resulting from the blockage of central Y_1 receptors in haemorrhage-shocked rats and (2) the involvement of the histaminergic system in this action.

KEY WORDS
histamine, neuropeptide Y, BIBP 3226, haemorrhagic shock

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STRESZCZENIE

WSTĘP: Pobudzenie ośrodkowego układu histaminergicznego wywołuje działanie resuscytacyjne w modelu wstrząsu krwotocznego, podczas gdy neuropeptyd Y (NPY) podawany do komory bocznej mózgu (icv) powoduje efekt depresyjny w stanie hipotensji krwotocznej. Mając na uwadze, iż na neurony histaminergiczne jądra guzowo-suteczkowatego mają wpływ neurony wytwarzające NPY zlokalizowane w jądrze olbrzymiokomórkowym tylnym podwzgórza, celem pracy było zbadanie (1) efektów krążeniowych antagonisty receptorów typu 1 dla NPY (Y_1) we wstrząsie krwotocznym oraz (2) możliwego udziału układu histaminergicznego w tych efektach.
MATERIAŁ I METODY: Badania przeprowadzono u szczurów samców szczepu Wistar w znieczuleniu ogólnym przy użyciu ketaminy i ksylazyny, u których wywoływano nieodwracalny wstrząs krwotoczny ze średnim ciśnieniem tętniczym (MAP) 20–25 mmHg oraz wskaźnikiem przeżycia 2 h 0%. Niezwłocznie po indukcji wstrząsu dokonywano premedykacji icv antagonistami receptorów histaminowych H_1 i H_2 chlorfenyraminą (50 nmol) i ranitydyną (50 nmol), antagonistą/odwrotnym agonistą receptorów $H_{3/4}$ tioperamidem bądź 0,9% roztworem NaCl. Po pięciu minutach zwierzętom podawano icv antagonistę receptorów Y_1 BIBP 3226 (64 nmol/kg).
WYNIKI: BIBP 3226 wywoływał wzrosty MAP, ciśnienia tętna (PP) oraz nerkowego przepływu krwi (RBF), powodował także wzrost do 100% wskaźnika przeżycia 2 h. Chlorfenyramina hamowała zmiany MAP, PP i RBF wywoływane przez BIBP 3226 oraz zmniejszała do 0% wskaźnik przeżycia 2 h. W odróżnieniu do tego ranitydyna i tioperamid nie wykazywały wpływu na działanie BIBP 3226.
WNIOSKI: Zablokowanie ośrodkowych receptorów Y_1 wywołuje efekt presyjny u szczurów we wstrząsie krwotocznym. Działanie to odbywa się przy udziale układu histaminergicznego.

SŁOWA KLUCZOWE

histamina, neuropeptyd Y, BIBP 3226, wstrząs krwotoczny

INTRODUCTION

There are many neuronal systems involved in central cardiovascular regulation in haemorrhagic hypotension. Centrally acting neurotransmitters and neuromodulators can be functionally divided into two groups [1]. Proopiomelanocortin (POMC)-derived peptides, acetylcholine, cholecystokinin, thyrotropin-releasing hormone and histamine are able to increase – acting directly or indirectly – the activity of rostral ventrolateral medulla (RVLM) neurons in shock, whereas opioid peptides induce an opposite effect [1,2,3]. Histaminergic neurons are located in the tuberomammillary nuclei of the hypothalamus and send innervations to almost all parts of the brain [4]. Thus, they are able to influence many functions of the central nervous system, including cardiovascular regulation [5]. In normotension, exogenous histamine administered to the brain lateral ventricle in anaesthetized rats induces a dose-dependent pressor effect with tachycardia [3]. These effects are even more pronounced in critical haemorrhagic hypotension [3]. In these conditions, activation of the central histaminergic system leads to the reversal of haemorrhagic shock in rats as a result of activation of the sympathetic [6] and renin-angiotensin systems [7], as well as secretion of arginine vasopressin [8] and POMC-derived peptides [9].

Neuropeptide Y (NPY) is a 36-amino acid peptide present in the central and peripheral nervous systems [10]. It belongs to the polypeptide family which also includes peptide YY (PYY) and pancreatic polypeptide (PP). All these peptides act via at least five subtypes of receptors, classified as NPY receptors (Y_1, Y_2, Y_4, Y_5 and Y_6). There is no consensus on the role of NPY in central cardiovascular regulation. Experimental studies demonstrate both pressor [11] and depressor effects [12] after central NPY administration, depending on the experimental model, animal strain, anaesthesia, and the route of administration. Our preliminary data demonstrated a depressor effect resulting from icv administration of NPY in haemorrhage-shocked rats [13].

Since histaminergic neurons of the tuberomammillary nucleus receive input from neurons producing NPY localized in the caudal magnocellular nucleus of the hypothalamus [14], the aim of the present study was to examine (1) the cardiovascular effects of the Y_1 receptor antagonist in haemorrhagic shock and (2) the possible involvement of the histaminergic system in the action. The results were presented as a poster presentation during the 43rd Annual Meeting of the European Histamine Research Society (Lyon, France) [13].

MATERIAL AND METHODS

All the procedures were performed in accordance to EU directives and reviewed by the Local Ethics Committee, Katowice, Poland (Notification No 32/2014). The studies were performed in male Wistar rats weighing 260–310 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%), and provided with food and water ad libitum.

After inducing general anaesthesia with ketamine/xylazine (100 mg/kg + 10 mg/kg intramuscularly, supplemented if required), the rats were implanted with catheters filled with heparinised saline (300 IU/ml) in the right femoral artery and vein. Mean arterial pressure (MAP) and heart rate (HR) were measured using a TAM-A transducer amplifier module and ECGA amplifier (Hugo Sachs Elektronik, Germany), respectively. An electromagnetic perivascular probe (type 1RB, Hugo Sachs Elektronik, Germany) was implanted around the right renal artery to monitor renal blood flow (RBF) using a Transit Time Flowmeter TTFM Type 700 (Transonic Systems Inc., USA). All measurements of blood flow were started after a 30 min adaptation period to avoid influences of probe implantation.

For icv treatment, the 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 [3]. All icv injections were made in the volume of 5.0 µl. Correctness of the injections was verified as previously described [3].

Severe haemorrhagic shock, according to the method by Guarini et al. [15], was produced by intermittent blood withdrawal from the catheter inserted into the right femoral vein over a period of 15–25 min, until MAP decreased to and stabilised at 20–25 mmHg.

Immediately after inducing critical MAP, the animals were pre-treated icv with the histamine H_1 and H_2 receptor antagonists chlorpheniramine (50 nmol) and ranitidine (50 nmol) as well as with the $H_{3/4}$ receptor antagonist/inverse agonist thioperamide (50 nmol), respectively, or saline. Five minutes later, the rats were injected icv with the NPY receptor type 1 antagonist – BIBP 3226 (64 nmol/kg). The doses of NPY and histamine receptor antagonists were taken from the literature [3,16]. 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 ± 0.5°C using heating lamps throughout the experiment. All the experiments were performed between 8.00 and 14.00.

According to the recommendations of the Local Ethics Committee, to avoid duplicating the experiments performed at our laboratory with the same rat strain, using the same experiment protocol and with the same histamine receptor antagonists and saline [3,17], we did not repeat experiments in the control saline icv-treated groups and cited and discussed previously published results.

The following drugs were used: BIBP 3226 (Tocris Bioscience, UK), chlorpheniramine maleate, ranitidine hydrochloride, thioperamide maleate (Research Biochemicals Incorporated, USA), ketamine hydrochloride, xylazine (Biowet Sp. z o.o., Poland), heparin (Polfa, Poland). All the drug solutions were prepared freshly on the day of the experiment.

All the values are given as means ± standard deviation, with $p < 0.05$ considered as the level of significance. Fisher's exact test was used to examine the statistical differences in survival percentage. Statistical evaluation of the measured cardiovascular parameters was performed using analysis of variance (ANOVA) and the Student-Newman-Keuls post-ANOVA test.

RESULTS

The initial pre-bleeding values of MAP (Fig. 1A), pulse pressure (PP) (Fig. 1B), HR (Fig. 1C) and RBF (Fig. 1D) did not reveal significant differences between the groups. The total bleeding volume necessary to induce critical hypotension in all the animals was 2.36 ± 0.42 ml/100 g body weight.

In the control saline-treated group, the induction of critical MAP (20–25 mmHg) was accompanied by a decrease in PP from 26.2 ± 5.1 mmHg to 10.2 ± 2.3 mmHg, HR from 344 ± 18 beats/min to 229 ± 21 beats/min and RBF from 5.32 ± 0.5 to 0.78 ± 0.16 ml/min [17]. There were no differences among any of the groups in post-bleeding values of MAP, PP, HR and RBF (Fig. 1A-D).

BIBP 3226 evoked long-lasting rises in MAP (Fig. 1A), PP (Fig. 1B) and RBF (Fig. 1D) as well as an increase in the survival rate of 2 h up to 100%, with no significant influence on HR (Fig. 1C).

MAP, PP and RBF changes induced by BIBP 3226 were inhibited by pre-treatment with chlorpheniramine, but not with ranitidine and thioperamide (Fig. 1A-B, D). In addition, pre-treatment with chlorpheniramine, led to a decrease in the survival rate to 16.7% ($p < 0.05$ vs. saline-pre-treated and BIBP 3226--injected group, Fisher's exact test).

As previously described, in the control saline-treated groups, chlorpheniramine had no effect on the measured cardiovascular parameters, whereas ranitidine and thioperamide given alone induced pressor effects in the used model of haemorrhagic shock [3].

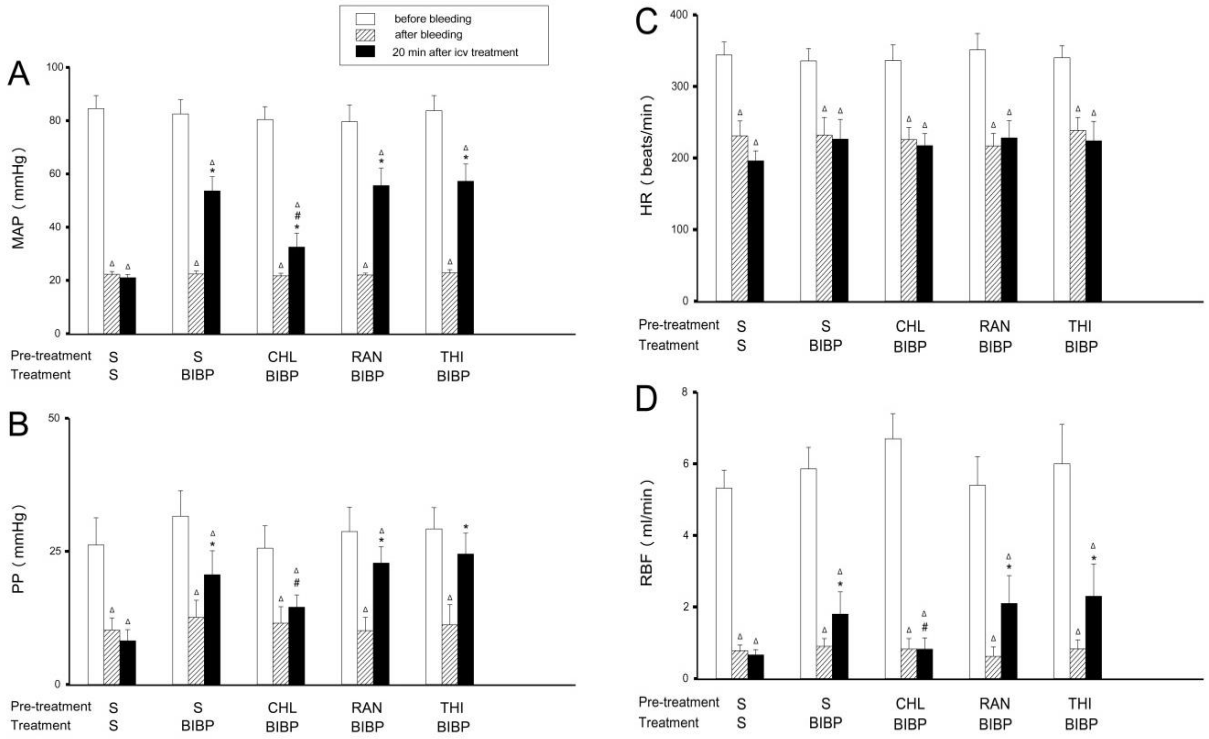

Fig. 1. Influence of icv pre-treatment with saline (S, 5 μl), chlorpheniramine (CHL, 50 nmol), ranitidine (RAN, 50 nmol) and thioperamide (THI, 50 nmol) on BIBP 3226 (64 nmol/kg, icv)-induced changes in MAP (A), PP (B), HR (C) and RBF (D) in haemorrhage-shocked rats; n = 6; mean ± SD; $^{\Delta}$ p < 0.05 vs. pre-bleeding value, * p < 0.05 vs. saline-injected group, $^{\#}$ p < 0.05 vs. saline-pre-treated followed by BIBP 3226-injected group; ANOVA, Newman-Keuls test.

Ryc. 1. Wpływ premedykacji icv 0,9% roztworem NaCl (S, 5 μl), chlorfenyraminą (CHL, 50 nmol), ranitydyną (RAN, 50 nmol) i tioperamidem (THI, 50 nmol) na zmiany MAP (A), PP (B), HR (C) i RBF (D) wywoływane przez BIBP 3226 (64 nmol/kg, icv) u szczurów we wstrząsie krwotocznym; n = 6; średnie ± SD; $^{\Delta}$ p < 0,05 vs. wartość sprzed krwotoku, * p < 0,5 vs. grupa, w której podawano 0,9% roztwór NaCl, $^{\#}$ p < 0,05 vs. grupa, w której podawano 0,9% roztwór NaCl, a następnie BIBP 3226; ANOVA, test Newmana-Keulsa.

DISCUSSION

The present results demonstrate for the first time a long-lasting pressor effect resulting from the blockage of central Y_1 receptors in haemorrhage-shocked rats. Moreover, we demonstrate the functional interaction between NPY and the central histaminergic system in regulating cardiovascular system function in haemorrhagic shock.

The used model of haemorrhagic shock belongs to models of irreversible shock, with critically low values of peripheral blood flow, reflex-induced bradycardia and death of all the control animals within 30 min [2,3]. As in the previous studies [18], we used this model to study the cardiovascular effects of the Y_1 receptor antagonist and histaminergic ligands because of good reproducibility and stable initial cardiovascular parameters.

NPY is synthesized mainly in the peripheral tissues, especially in the gastrointestinal tract and adrenal glands, and does not easily pass the blood-brain barrier. However, recent evidence suggests that circulating hormones are able to modulate brain mechanisms regulating blood pressure. The mechanism is associated with the function of circumventricular organs which are located in the walls of the brain ventricular system. These organs are characterized by a lack of blood-brain barrier [19]. The neurons of circumventricular organs express receptors for many circulating hormones, including NPY and histamine [19]. Therefore, the intraventricular route of administering these hormones may be used to demonstrate the influences of peripherally-borne hormones on the function of the central nervous system [19]. Interestingly, in many cases the central and peripheral effects of these hormones are completely different. For example, histamine decreases peripheral vascular resistance and blood pressure acting peripherally, while after central administration, it induces a long-lasting pressor effect [3]. The present results demonstrate for the first time the resuscitating effect of the Y_1 receptor antagonist given icv in haemorrhagic shock in rats. Y_1 receptors are also involved in central cardiovascular regulation in normotension. The studies by Cheng et al. [12] show that injecting NPY into the NTS of rats decreases

MAP and HR, and the Y_1 receptor antagonist BIBP 3226 and G_i/G_o-protein inhibitor (pertussis toxin), attenuate these hypotensive effects [12]. In addition, a selective Y_1 receptor agonist increases the expression of $ERK_{1/2}$, ribosomal protein S6 kinase (RSK) and the phosphorylation of eNOS [12]. These results demonstrate that the NPY-mediated depressor effect is mediated via Y_1 receptor-PKC-ERK-RSK-eNOS and Ca^{2+}-eNOS signalling pathways [12].

Particular mechanisms involved in BIBP 3226-evoked resuscitating action in critical hypotension are not clear. According to the literature, we suggest possible direct and indirect mechanisms since (1) NPY acting in RVLM may inhibit responses evoked by somatic inputs [20], (2) neurons located in the arcuate nucleus and projecting to the paraventricular nucleus (PVN) express NPY which is able to decrease MAP, HR and lumbar sympathetic activity [21] and (3) there are morphological and functional interactions between NPY and the histaminergic system in central cardiovascular regulation in normotensive rats [14, 22].

To determine the role of the histaminergic system in central BIBP 3226-induced resuscitating action, we used histamine receptor ligands given icv. The present results show that the H_1 antagonist is able to influence the BIBP 3226-mediated resuscitating effect in haemorrhagic shock. These data are in line with our previous studies in which we demonstrated the involvement of H_1 receptors in the central histamine-mediated resuscitating effect in haemorrhage-shocked rats [3]. Interestingly, Tanida et al. [22] demonstrated that the inhibitory effects of centrally injected NPY on renal sympathetic nerve activity and MAP in normotensive rats are blocked by icv pre-treatment with thioperamide, but not with diphenhydramine, an anta-gonist of the histamine H_1 receptor. We suggest that the differences can result from (1) completely dif-ferent initial cardiovascular conditions (normotension versus critical haemorrhagic hypotension) and (2) distinct neuronal pathways activated in response to blood loss and affected by the NPY in our study.

Although we demonstrated the involvement of the histaminergic system in BIBP 3226-mediated cardiovascular effects in shock, there are limitations of our study. Firstly, BIBP 3226 has been used to study the role of Y_1 receptors in many studies [16,23], however, it is able to block not only NPY Y_1, but also neuropeptide FF (NPFF) receptors (K_i values are 1.1 and 79 for rNPY Y_1 and $hNPFF_2$, respectively) [24] and therefore, we cannot exclude the role of NPFF in the demonstrated effects. Secondly, there are many central neuronal systems which are able to influence the cardiovascular centre function in addition to histamine and NPY. Finally, we do not present the region of BIBP 3226 action and particular histaminergic neuronal pathways involved.

In conclusion, the results of our studies demonstrate for the first time (1) the pressor effect of the centrally acting NPY Y_1 receptor antagonist in haemorrhage-shocked rats and (2) the involvement of the histaminergic system in this action.

Author's contribution

Study design – A. Krawiec, J. Jochem
Data collection – A. Krawiec, K. Jasikowska
Data interpretation – A. Krawiec, K. Jasikowska, J. Jochem
Statistical analysis – K. Chojnacka, A. Mitera
Manuscript preparation – J. Jochem, A. Krawiec
Literature research – K. Chojnacka, A. Mitera, J. Jochem

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